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SECTION MEETINGS

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University of Chicago	March 14, 1944
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14534

Negative Results in Studies of Epidemic Diarrhea, Nausea and Vomiting of Unknown Cause.

Hobart A. Reimann, Alison H. Price, and John H. Hodges.
From the Jefferson Medical College and Hospital, Philadelphia.

Because of the absence of any demonstrable bacterial cause, several investigators suspected the syndrome called epidemic diarrhea, nausea and vomiting to be caused by a filtrable virus acting either on the central nervous system[1] or on the gastrointestinal tract.[2] The nature of the infection, its probable air-borne transmission and its appearance in explosive outbreaks concurrently in widely scattered places supports this view. There is reason to believe that certain viruses may cause primary gastro-enteritis in man, but until recently[3,4] none was thought to be particularly enterotropic, except perhaps the virus of poliomyelitis.

A widespread epidemic in Philadelphia in November, which involved 20% of our student body, provided material for study.[5] Food poisoning was excluded. Bacteriologic examination of stools and pharyngeal secretions from 10 carefully studied patients revealed none of the usual bacteria or other parasites commonly associated with enteritis and no unusual ones. Diarrheal stool samples from 5 patients were spun at slow speed in the centrifuge, and the supernatant fluid was inoculated orally, intranasally, and rectally into young white mice. Samples of the same fluids were passed through Berkefeld N and medium Mandler filters and inoculated intracerebrally. Pharyngeal washings in bouillon from the same patients were inoculated intranasally; and after filtration, intracerebrally. About 100 mice were used in the tests but only a few became sick and died. At necropsy several of them had pneumonitis, but no other significant lesions were observed even after microscopic study of sections of the viscera. Suspensions of the lungs and spleen from several mice were reinoculated by various routes into 100 fresh mice, in a few trials to the fourth passage, without any consequence of note. Three samples of original filtered fluid from stools were inoculated into the chorio-allantoic membrane of developing chick embryos but again without results.

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[1] Rischel, A., *Ugeskr. Laeg.*, 1935, **97**, 1285; Henningsen, E. J., *Ugeskr. Laeg.*, 1936, **98**, 45; Bradley, W. H., *Brit. Med. J.*, 1943, **1**, 309; Miller, R., and Raven, M., *Brit. Med. J.*, 1936, **1**, 1242.

[2] Boardman, W. W., *Am. J. Med. Sci.*, 1939, **196**, 833.

[3] Silva, M., *Hospital*, Rio de Janeiro, 1940, **18**, 1015; Baker, J. A., *J. Exp. Med.*, 1943, **78**, 435.

[4] Light, J. S., and Hodes, H. L., *Am. J. Pub. Health*, 1943, **33**, 1451.

[5] Reimann, H. A., Price, A. H., and Hodges, J. H., *Epidemic Diarrhea, Nausea and Vomiting of Unknown Cause*, to be published.

In December, Light and Hodes[4] reported the isolation of a filtrable agent from the stools of infants suffering from a well-known epidemic form of diarrhea of the newborn, with which they regularly produced enteritis and diarrhea in calves by intranasal inoculation. The agent was indeed an unusual one which was not regularly inactivated by boiling for 5 minutes or by heating at 70°C for 1 hour, yet it appeared to be the causative agent since it was neutralized by the serums of 6 convalescent babies.

After this publication appeared we applied the technic described using 4 stool specimens which had been preserved frozen with solidified carbon dioxide since November (2 months) and specimens from 4 patients with sporadic attacks in January. The stools were thawed, the supernatant fluids were immediately passed through medium Mandler filters and inoculated in 10 cc amounts intranasally into 8 4-week-old calves. The calves were observed for 2 months but all except one remained well. One became sick for 3 days without diarrhea a month after inoculation and died, but at necropsy no gross lesions were seen.

Summary. Attempts to isolate a filtrable infectious agent from adult patients with epidemic diarrhea, nausea, and vomiting were unsuccessful. We were unable to duplicate the results of Light and Hodes. Perhaps the virus had disappeared during preservation of the frozen stools, or was never present in the stools, or insufficient amounts of inoculum were used, or the disease in adults with which we dealt is different from that in the newborn.

14535

A Potentiated Chemotherapeutic Action of Sulfathiazole and Quinine.*

B. K. HARNED, RUTH E. MILLER, MARIA WIENER, AND NELLIE P. WATTS.

From the Departments of Pharmacology and Bacteriology, Woman's Medical College of Pennsylvania, Philadelphia.

In the course of an investigation on the effects of the simultaneous administration of antimalarials and sulfonamides we observed that quinine and sulfathiazole appeared to exert in mice a potentiated chemotherapeutic action against an infection with a beta hemolytic streptococcus, strain 1048M. Stuart, Powell and Bibbins[1] reported that against a streptococcal infection in mice the chemotherapeutic action of quinine sulfanilamide 3HCl was equal to but not greater than that of sulfanilamide. All other cinchona-sulfanilamide compounds tried by these investigators were less effective than sulfanilamide. Other workers, Miura,[2] Jacobi and Coenen,[3] Sattler and

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* The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Woman's Medical College of Pennsylvania.

[1] Stuart, E. H., Powell, H. M., Rose, C. L., and Bibbins, F. E., *J. Am. Pharm. Assn.*, 1939, **28**, 90.

[2] Miura, K., *Jap. J. M. Sc., IV, Pharm.*, 1940, **12**, 209.

[3] Jacobi, J., and Coenen, F., *Munchen. Med. Wchnschr.*, 1940, **87**, 723.

TABLE I.
The Protective Action of Sulfathiazole With and Without Quinine Against Hemolytic Streptococcal Infections* in Mice.

Group	No. mice	No. survived	% survived	Standard error$_p$	D/S.E.$_{D_p}$	Chances in 100 of a true difference between groups I and II
I. Sulfathiazole and quinine	71	54	76	5.07	2.38	99.1
II. Sulfathiazole	72	41	57	6.09		
III. Quinine	73	2	3			
IV. No drug	73	7	10			

* *Streptococcus hemolyticus*, strain 1048 mucoid, Lancefield group A, was the organism used for infection.

The drugs were administered for 48 hours prior to and for 168 hours subsequent to the infection. All of the survivors were placed on drug-free Purina checkers for an additional 168 hours. The survivors recorded in the table represent the number of mice living at the end of the second 168-hour period.

Tüchler,[4] and Schumacher,[5] have reported experimental and clinical data which suggest that combinations, or the concomitant administration of quinine and sulfanilamide or sulfapyridine are more effective that the sulfonamide alone.

In our experiments 289 mice were divided into 4 matched groups. Group I received sulfathiazole plus quinine ethyl-carbonate; group II, sulfathiazole; group III, quinine ethyl-carbonate; and group IV, no drug. The drugs were evenly dispersed by thorough trituration with ground Purina dog chow checkers and were fed *ad libitum*. The feeding started 48 hours before infection with the streptococci.

The number of mice shown in Column 2 of Table I represent 9 different experiments. In each of these experiments the 4 different groups were always run concurrently and the number of animals in each group in a given series was always the same. The concentration of sulfathiazole in groups I and II was the same in a given series but in the different series the concentration ranged from 0.025 to 0.3%. The concentration of the quinine ethyl-carbonate was always 0.3%. This form of quinine is almost tasteless to the human and apparently was not objectionable to the mouse.

Each mouse was injected intraperitoneally with 0.5 cc of a 1-500,000 dilution of an 18-hour blood broth culture of *Streptococcus hemolyticus*, strain 1048 M. Plate counts of the dilution before the injections showed that each animal received approximately 2500 organisms. The mice were kept on the drug for 7 days subsequent to the infection and the survivors were placed on drug-free Purina checkers for an additional 7 days.

The survivors recorded in Table I represent the animals which survived the 14-day period. The animals in Groups III and IV usually died within the first 24 hours. In Groups I and II 5 and 6 animals, respectively, survived the first 7 days but died before the 14th day. When the data from the 2 periods are analyzed in terms of the ratio of the difference to the Standard Error of the difference the chances that the difference between Groups I and II is true are 99 per 100 for each period. When the chi-square test is applied the chances that there is no difference between Groups I and II are 1.6 and 1.7 per 100 for the 2 periods. These analyses indicate that the probability of a true potentiated chemotherapeutic action of quinine and sulfathiazole is high.

Summary. Tests on 289 mice infected with *Streptococcus hemolyticus*, strain 1048M, gave evidence, with a reasonably high probability of certainty, that quinine and sulfathiazole exerted a potentiated chemotherapeutic action.

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[4] Sattler, A., and Tüchler, K., *Wien. Med. Wchnschr.*, 1940, **90**, 595.

[5] Schumacher, G., *Munchen. Med. Wchnschr.*, 1940, **87**, 1302.

14536

Effect of Feeding of "Soya Lecithin"* on Serum Cholesterol Level of Man.

Alfred Steiner and Beatrice Domanski. (Introduced by David Seegal.)
From the Research Service, First Division, Goldwater Memorial Hospital, Department of Hospitals, and College of Physicians and Surgeons, Columbia University, New York City.

In a previous report[1] it was postulated that the hypercholesterolemia resulting from the feeding of egg yolk powder, which is rich in cholesterol and lecithin, was due partially to its lecithin content. It was therefore considered of interest to determine the effect of feeding "soya lecithin" on the serum cholesterol level of 8 human subjects.

Method. Twenty-five grams of "soya lecithin"* were fed daily to 7 patients for 6-week periods and to one patient (No. 5) for a 10-week period. The "soya lecithin" was fed in 5 g portions spread between two crackers. The diagnosis in 6 of the 8 patients was coronary arteriosclerosis, made on the basis of clinical symptoms, signs and electrocardiographic changes. Five of the 6 individuals had previously experienced a myocardial infarction. The remaining 2 patients had classical rheumatoid arthritis, the diagnosis of which was confirmed by roentgenographic change. No untoward symptoms or signs occurred during the "soya lecithin" feeding periods. Serum cholesterol determinations by the method of Bloor, Pelkan, and Allen[2] were made twice weekly during an initial 4-week control period, the 6-week "soya lecithin" feeding period and a subsequent 4-week control period. Throughout these observations, the patients were fed a standard hospital diet. This was not rigidly controlled. It contained approximately 90 g of protein, 100 g of fat, and 340 g of carbohydrate, with a caloric equivalent of 2600 calories. Four of the patients were fed "soya lecithin" for a second test period of 6 weeks. Basal metabolic rates were determined during the control period, and in the 4th and 5th week of the "soya lecithin" feeding period.

Results. The results of feeding "soya lecithin" on the serum cholesterol level are contained in Table I. There were 12 feeding periods in 8 patients. It can be seen that the serum cholesterol level declined during each period of "soya lecithin" feeding. The extent of the fall from the average of control period determinations varied from 44 to 144 mg/100 cc. Fig. 1 demonstrates, in one instance, the lowering effect of "soya lecithin" on the serum cholesterol level. The serum cholesterol, however, returned to the control levels, in each instance, after 4 to 5 weeks, even though the "soya lecithin" feeding was continued. After further control observations, 4 of the patients were fed "soya lecithin" during a second period. The results obtained were similar to those in the initial experiment. Basal metabolic rates remained approximately the same during the control period and the period of maximum depression of the serum cholesterol level.

Discussion. From the above it is evident that the feeding of "soya lecithin" to human subjects results in a lowering of the serum cholesterol level. Corwin[3] has reported that a hypercholesterolemia was produced in dogs by the feeding of lecithin derived from the adrenal gland. However, Kesten and Silbowitz[4] have stated that the feeding of "soya lecithin" inhibits the hypercholesterolemia of rabbits fed a diet rich in cholesterol. The effect of "soya lecithin" and that of lecithin obtained from the adrenal gland on serum cholesterol level are apparently different. It should be emphasized that "soya lecithin" is

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[1] Steiner, A., and Domanski, B. N., *Am. J. Med. Sc.*, 1941, **201**, 820.

* American Lecithin Co., Elmhurst, L.I. Approximate composition, lecithin 20%, cephalin 20%, oil 30%, phytosterols 2%, inositol and allied compounds 15%, carbohydrates 10%.

[2] Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, **52**, 191.

[3] Corwin, W. C., *Arch. Path.*, 1938, **26**, 426.

[4] Kesten, H. D., and Silbowitz, R., Proc. Soc. Exp. Biol. and Med., 1942, **49**, 71.

TABLE I.
Effect of Soya Lecithin Feeding on Serum Cholesterol Level of 8 Patients.
Serum cholesterol expressed as mg/100 cc.

Patient No.	Initial control period (4 wks) Range	Avg	Soya lecithin period (6 wks) Lowest value	Ext. of decline	2nd control period (4 wks) Range	Avg
1	329-378	349	290	59	309-357	340
2	537-561	546	402	144	458-536	496
3	458-505	474	388	86	442-462	456
4a	320-386	348	275	73	315-345	328
b	345-380	365	302	63	371-400	384
†5a	242-252	245	192	53	238-258	245
b	239-256	245	195	50	236-256	242
*6a	226-240	235	190	45	215-245	235
b	240-252	245	201	44	253-275	262
*7	232-238	235	180	45	220-238	232
8a	416-424	420	344	84	395-426	410
b	390-420	410	335	75	416-449	430
				Avg 68		

* Patients with rheumatoid arthritis.
a = First experimental period.
b = Second ” ”
† Lecithin feeding period—10 weeks.

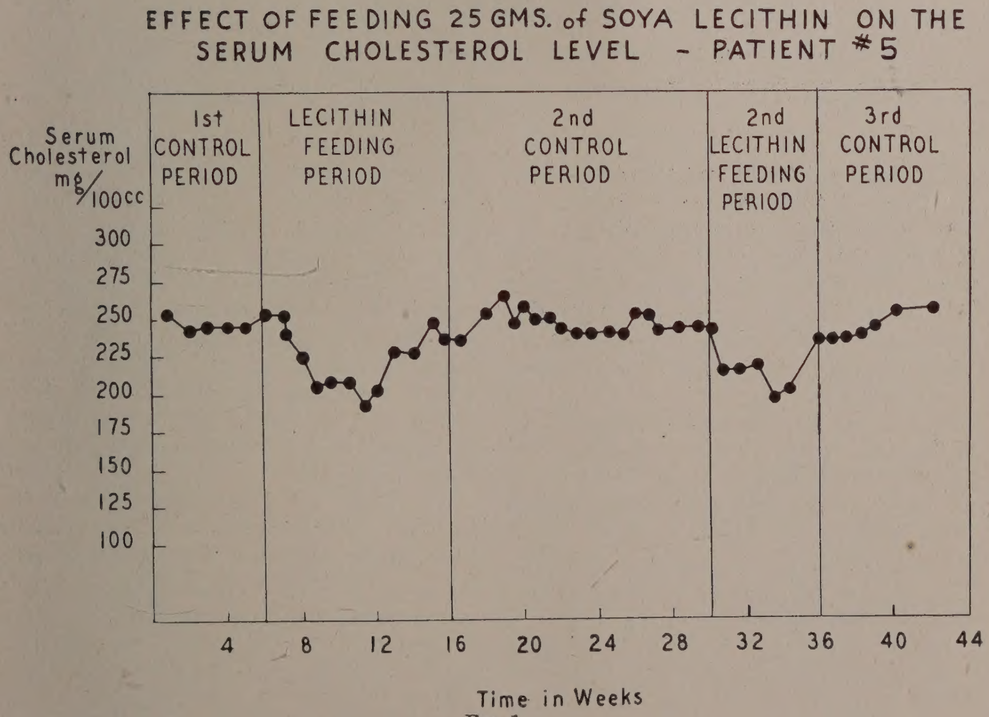

Fig. 1.

composed not only of lecithin, but also of cephalin, oil, phytosterol, inositol, and carbohydrates as well.

The inference that the hypercholesterolemia resulting from the feeding of egg yolk powder was partially due to its lecithin content[1] is not supported by these experiments. On the contrary, "soya lecithin" has been found to lower the serum cholesterol level not only in people on a normal diet but also in rabbits on a high cholesterol regime.[4]

Since this article was prepared Gross and Kesten[5] have reported that the feeding of "soya lecithin" was effective in reducing the hypercholesterolemia in all of 3 patients. The diagnosis in 2 of these individuals was xanthomatosis. Necrobiosis lipoidica diabeticorum was the stated diagnosis in the third patient. Adlersberg and Sobotka[6] have also shown that the elevated serum cholesterol of 5 patients with xanthomatosis was lowered by "soya lecithin" feeding.

Summary. (1) Twenty-five grams of "soya lecithin" were fed to 8 patients for 6-week periods. (2) The serum cholesterol level in each instance was lowered significantly. The decline was maintained for only 5 weeks despite the maintenance of the "soya lecithin" regime. (3) The induced hypocholesterolemia was not associated with an increase in the basal metabolism.

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[5] Gross, P., and Kesten, B., *Arch. Derm. and Syph.*, 1943, **47**, 159.

[6] Adlersberg, D., and Sobotka, H., *J. Mt. Sinai Hosp.*, 1943, **9**, 955.

14537 P

Effect of Adrenotropic Hormone on Ascorbic Acid and Cholesterol Content of the Adrenal.*

GEORGE SAYERS, MARION A. SAYERS, HELEN L. LEWIS, AND C. N. H. LONG.
From the Department of Physiological Chemistry, Yale University School of Medicine, New Haven, Conn.

In a previous report (Sayers, Sayers, White, and Long[1]) it was shown that a single dose of pure adrenotropic hormone markedly depletes the cholesterol content of the adrenal to about 50% of its normal value in the space of 3 hours. The alterations in the ascorbic acid content of the adrenal that follows the administration of adrenotropic hormone have now been determined.

The animals used were 24-day-old male rats of the Yale strain. They were obtained from the Biochemical Laboratory of the Connecticut Agricultural Experiment Station through the courtesy of Dr. Rebecca Hubbell. Pure adrenotropic hormone was prepared according to the method of Sayers, White and Long.[2] The hormone was injected intraperitoneally as an aqueous solution in a volume of 0.5 ml. At the end of the experimental period the animals were anesthetized with nembutal, the adrenals removed and weighed to the nearest 0.1 mg on a torsion balance. The ascorbic acid content of the adrenals was determined by the method of Bessey[3] and the cholesterol content by the method of Schoenheimer and Sperry as described by Sperry.[4]

The changes which take place in the ascorbic acid and cholesterol content of the adrenals following the injection of a single dose of pure

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* This investigation has been aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

[1] Sayers, G., Sayers, M. A., White, A., and Long, C. N. H., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 200.

[2] Sayers, G., White, A., and Long, C. N. H., *J. Biol. Chem.*, 1943, **149**, 425.

[3] Bessey, O. A., *J. Biol. Chem.*, 1938, **126**, 771.

[4] Sperry, W. M., *Am. J. Clin. Path., Tech. Suppl.*, 1938, **2**, 91.

TABLE I.
Effect of Adrenotropic Hormone on Cholesterol and Ascorbic Acid Content of Adrenals of 24-day-old Rats.

Interval between administration of adrenotropic hormone (4 mg/100 g body wt) and removal of adrenals	Adrenal cholesterol		Adrenal ascorbic acid	
	No. rats	mg per 100 mg fresh tissue	No. rats	mg per 100 g fresh tissue
Controls	45	3.03 ± 0.08*	8	314.2 ± 6.1
20 minutes	—	—	5	212.6 ± 6.1
1 hour	6	2.62 ± 0.24	5	134.2 ± 6.9
3 ”	27	1.57 ± 0.07	5	141.3 ± 11.3
6 ”	8	1.71 ± 0.08	5	237.0 ± 15.2
9 ”	8	1.80 ± 0.11	5	293.9 ± 6.1
12 ”	4	1.98 ± 0.24	4	327.7 ± 16.6
24 ”	7	3.06 ± 0.19	2	351.4

*Mean and standard error.

adrenotropic hormone are shown in Table I, The cholesterol data are an extension of those previously reported.[1]

It will be observed that the hormone produces a prompt decrease in the ascorbic acid content of the adrenal so that within 20 minutes the level is reduced to two-thirds its initial value. The maximum decrease is reached in 1 hour but it remains at a low level for 2-3 hours and returns to its initial level some 9 hours later. Twenty-four hours after the injection of hormone the ascorbic acid concentration may be slightly greater than normal. The decrease in adrenal cholesterol is at a slower rate, the maximum being reached in 3 hours and it does not return to normal until a period somewhere between 12 and 24 hours after hormone administration. It appears that both the initial depletion and subsequent recovery occur sooner in the case of ascorbic acid than of cholesterol.

The marked change which takes place in adrenal cholesterol following the administration of adrenotropic hormone suggests that this substance is concerned with the synthesis of cortical hormone. The present findings indicate that ascorbic acid is also involved in this process. Giroud *et al.*[5] have already reported that cortical hormone synthesis is dependent upon the presence of vitamin C in the adrenals. Comparable studies are now being made in the guinea pig, an animal which cannot synthesize ascorbic acid.

Summary. A single dose of pure adrenotropic hormone diminishes both the ascorbic acid and cholesterol content of the adrenals. The level of ascorbic acid is reduced to two-thirds within 20 minutes and one-half of its normal value in one hour. It has again returned to its initial concentration 9 hours after hormone administration. On the other hand the maximum decrease in cholesterol is not reached until 3 hours after injection and its restoration also occurs at a slower rate. It is suggested that the two processes may be related to cortical hormone synthesis in or release from the adrenal gland.

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[5] Giroud, A., Santa, N., Martinet, M., and Bellon, M. T., *C. R. soc. biol.*, 1940, **134**, 100.

14538

An Experimental Study of Monopolar and Bipolar Derivations in the Human Electroencephalogram.*

ROBERT COHN. (Introduced by C. E. Leese.)

From the Blackburn Laboratory, St. Elizabeth's Hospital, Washington, D.C.

A prominent feature of the normal human electroencephalogram, in transverse bipolar derivations, is the large amplitude of the alpha waves obtained from the occipital regions as compared to those from the frontal and parietal regions. Another outstanding characteristic of the human EEG is the generally higher amplitude of the wave-forms from the frontal and parietal leads in "monopolar," as compared to "bipolar" derivations from the same leads.[1] The latter property is most marked when the reference electrodes for the monopolar leads are placed on the ear lobes or in the mastoid regions. Experiments were devised to determine the basic factors responsible for these phenomena.

Method. Cadavers were used in all experiments. The post-mortem interval varied from 3 hours to 4 days. The variation in elapsed time from death to experiment had no important influence on the uniformity ot the results. A small trephine hole was made in the skull. The dura was then split and shielded copper wires, sealed in glass† were inserted through the skull opening into the brain substance to a depth of from 1 to 3 cm. The depth of insertion, between the ranges noted, was in no way critical. The source of artificial potential differences was obtained from a Hewlett Packard type 200D audio oscillator. The output voltage of the oscillator was regulated to give an approximately 2 mm amplitude deflection when recording from the transverse frontal leads. The oscillator voltage necessary to give the above deflection (with standard amplifier gain) was of the order of 50 milli-volts. The voltage was maintained at the proper determined value throughout the experiment. The resistance across the stimulus leads in contact with the brain substance was estimated to be 1 megohm. The voltage changes on the scalp surfaces were picked-up by small solder electrodes placed along symmetrical parasagittal lines over each cerebral hemisphere, generally in the frontal, parietal, and occipital regions. All monopolar leads were taken with either the ear lobes or the mastoid regions as reference points. Graphic recordings were made with an A.M. Grass 3 channel ink-writing oscillograph. Resistance measurements were made with an RCA Junior Voltohmist.

Results. Early in the investigation it became clear that with an artificial electric dipole in the occipital region a non-linear attenuation of *amplitude* resulted when the pick-up electrodes (bipolar) were linearly spaced 1,5,10,15 . . . cm from the region of stimulation. This phenomenon was most distinct when the pick-up electrodes were placed over the convex surface of the head. This non-linear attenuation is shown in the top 3 lines of Fig. B.

The marked similarity in amplitude characteristics between the normal human EEG in the transverse derivations (top 3 lines of Fig. A) and that of the artificially generated EEG from similar leads (top 3 lines of Fig. B) is obvious.

When the oscillating voltage was placed in the frontal region the findings were reversed, that is the amplitude was greatest in the frontal region. Hence, it is apparent that the amplitude of the waves picked-up by the bipolar technic is a non-linear function of the distance of derivation from the region of disturbance.

Monopolar derivations (with respect to the

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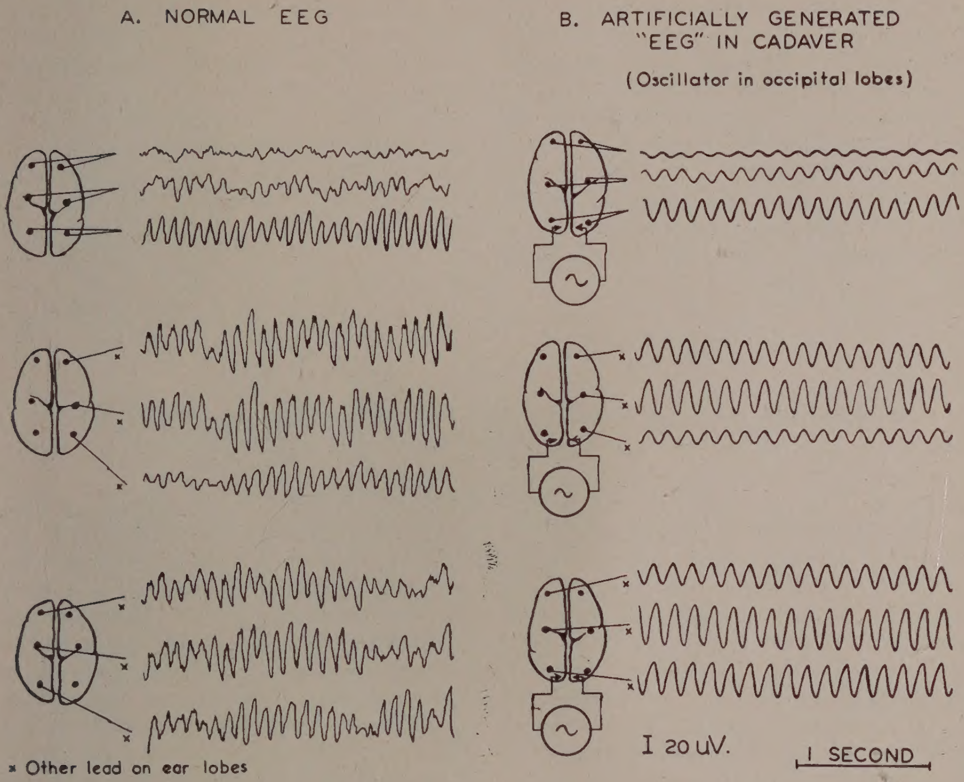
* This work was aided by the Supreme Council, 33° Scottish Rite, Masons of the Northern Jurisdiction.

[1] Rubin, M. A., *J. Neurophysiol.*, 1938, **1**, 313.

† The protruding edges of the sealed wires were cut flush and ground close to the edge of the glass in order to keep the area of direct stimulation as restricted as possible.



ear lobes) when using artificial electric dipoles, invariably show wave-forms of relatively large amplitude in all leads. This phenomenon is independent of the locus of the artificial source of potential difference (whether right, left, or middle in the frontal, parietal, or occipital regions). The lower 2 sets of simultaneous recordings of Fig. B are monopolar derivations with the stimulus dipole in the right occipital region. The similarity of these wave-forms to those seen in the normal human EEG (lower 6 lines of Fig. A) is remarkable. The minor variations of amplitude in the monopolar leads of the artificial system appear to be the result of local resistances.

Discussion. The marked similarity between the "spontaneous" and the artificial EEGs strongly suggests that the monopolar-bipolar amplitude differences observed in the normal human EEG are not primarily conditioned by the brain physiology; but are more probably conditioned by the attenuating properties of the anatomical components of the head and brain.

An electric dipole placed in the occipital regions is capable of giving sequences of waves that are similar, at least in amplitude characteristics, to those observed in the normal human EEG, using similar electrode placements. This fact lends strong support to the early hypothesis of Adrian[2] that the alpha rhythm of the EEG is primarily generated in the occipital lobes. Hence, even though it has been conclusively shown that other regions of the intact brain[3] as well as segments of brain tissue[4] under special conditions, will produce sequences of quasi-periodic potential oscillations, the important fact appears to be that the main amplitude component of the usual clinical EEG can be duplicated by the spread of focal occipital potentials.

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[2] Adrian, E. D., and Yamagiwa, D., *Brain*, 1935, **58**, 323.

[3] Kornmuller, A. E., *Die Bioelektrischen Erscheinungen der Hirnrindfelder*, Leipzig, George Thieme, 1937.

[4] Libet, B., and Gerard, R. W., *J. Neurophysiol.*, 1939, **2**, 153.

Conclusions. It has been shown that the important characteristic *amplitude properties* of the alpha component of the normal human EEG, in the usual monopolar and bipolar derivations primarily depend on the attenuating properties of the tissue through which the potential difference is transmitted. Moreover, noting the correspondence between waveforms seen in the normal EEG and those produced by an artificial occipital electric dipole in the cadaver, it is clear that the spread of an occipital electric disturbance is an excellent model for the production of the amplitude characteristics of the basic alpha component of the normal EEG.

14539 P

Serial Studies of Oxygen and Carbon Dioxide Content of Human Cord Blood.

Wm. J. Dieckmann and Sylvia Kramer.

From the Department of Obstetrics and Gynecology, The University of Chicago and The Chicago Lying-In Hospital.

The reports of human cord blood studies are based on one sample obtained either after vaginal or abdominal delivery. Several investigators obtained blood at cesarean section but so far as we know no one has described a method which permits repeated sampling of fetal blood.

Haselhorst and Stromberger[1] delivered the umbilical cord in 8 cesarean sections, performed under spinal anesthesia on patients not in labor, and stated that the oxygen content of both arterial and venous blood was less than that found after vaginal delivery. They attributed the increased oxygen content to the uterine contractions forcing more maternal blood through the placenta. Their average figures are given in Table I.

Eastman's[2] average figures for blood obtained after vaginal delivery are also listed. He delivered the cord in one cesarean section, clamped off a segment from which arterial and venous bloods were obtained.

We find it feasible in most cesarean sections to deliver the umbilical cord through a small incision in the uterus and either inject drugs into the fetal circulation or withdraw repeated specimens of fetal blood. Since most of our cesarean sections are performed under local anesthesia, ideal conditions are available for obtaining serial specimens of fetal blood with the fetus still *in utero*. We have drawn 2 to 3 cc of blood from the umbilical vein and artery. No more than 30 cc of blood should be taken from term babies and much less from premature fetuses. The time interval in these experiments was 5 minutes, but in some unreported studies of placental permeability it was 15 minutes.

An interval of 30 minutes is probably the maximum because further prolongation of the operation increases the danger of maternal infection. Furthermore, in operations performed under local or continuous caudal anesthesia there is a mental strain due to the operation and the uncomfortable position on the operating table.

The fetal heart rate must be counted frequently either by use of a sterile stethoscope or by palpation of the umbilical arteries. If the rate decreases below 100 per minute the baby's head is delivered, 0.3 cc solution posterior pituitary are injected into the uterine wall and after one minute the fetus is slowly delivered. After the uterus has contracted the cord is

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[1] Haselhorst, G., and Stromberger, K., *Z. f. Geburt. u. Gynäk.*, 1932, **102**, 16.

[2] Eastman, N., *Bull. Johns Hopkins Hosp.*, 1930, **4**, 221.

TABLE I.

				No. 1-K		No. 2-M		No. 3-G	
	1	2	3	Control	After oxygen	Control	After oxygen	Control	After oxygen
Cord—Vol. %									
Vein—O_2 content	3.53	10.4	10.5	6.13	8.57	6.8	12.6	12.79	14.73
Saturation %			50.5	30.8	43.1	37.0	69.0	64.3	74.0
CO_2 content			45.9	46.53	43.10	44.55	41.3	42.2	39.10
Artery—O_2 content	0.94	3.40	3.3	2.14	2.83	2.6			
Saturation %			15.8	10.8	14.2	14.0			
CO_2 content				50.13	48.55	50.1			
O_2 capacity			20.8	19.85		18.2		19.9	
Maternal—Vol. %									
Vein—O_2 content			11.0	8.51		10.4		8.10	10.40
Saturation %			71.3	70.7		70.0		54.0	68.5
CO_2 content			43.3	46.04		40.7		40.52	39.24
O_2 capacity			15.4	12.03		14.9		14.8	

Haselhorst and Stromberger—2. Average vaginal. 1. Average cesarean delivery.
Eastman—3. Average vaginal delivery.

stripped and clamped close to the fetus. This technic ensures a normal fetal blood volume. The blood for oxygen and carbon dioxide content is obtained with oiled syringes using 22-gauge needles. The usual amount of neutral potassium oxalate is used. The blood samples are kept in an ice bath and the analyses are completed as quickly as possible.

Data for the oxygen content of fetal blood before and after administration of 100% oxygen to the mother for five minutes are given in Table I and demonstrate the practicability of the method. Appreciable increases in the oxygen content are noted in the umbilical vein blood (arterial). The 3 patients were not in labor.

Data for the CO_2 content are also given. The Van Slyke and Neill constant volume apparatus was used. The oxygen capacity was calculated from the hemoglobin which was determined by the carbon monoxide method of Palmer. Eleven patients have been studied. Every experiment cannot always be completed; hemostasis of the uterine wall is at times impossible, the cord may become compressed, cord blood clots rapidly, etc.

Summary. A method is described by which repeated samples of human cord blood can be obtained over a short period of time. Undue prolongation increases the risk of the operation. The oxygen content of fetal blood is appreciably increased by the administration of oxygen to the mother.

14540

Phosphatase Activity in Chromatolytic Nerve Cells.*

DAVID BODIAN AND ROBERT C. MELLORS. (Introduced by H. A. Howe.)

From the Poliomyelitis Research Center, Department of Epidemiology, School of Hygiene and Public Health, Johns Hopkins University.

In nerve cells with axons extending into peripheral nerves, it is possible by simple nerve section to produce profound alterations in cytoplasmic structure and activities without causing cell destruction. These changes are fairly constant in their time course when pro-

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* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

duced in this manner, and are generally completely reversible, so that they may be studied at any given time of the cycle in a readily controllable fashion. Such an experimental preparation therefore lends itself not only to study of a fundamental neuropathological process but also to analysis of controllable changes in the basic physiological properties of the cell.

As a consequence of axon section there is initiated a series of morphological changes in the cell body. These retrograde changes, the "axon reaction," reach a climax after one or 2 weeks, when active axon regeneration is in process. The cellular reaction as seen in stained histological sections was first described by Nissl in 1892 and is commonly known as "chromatolysis," since the most obvious visible change is a loss of chromophilic or stainable material in the cytoplasm. This chromophilic material is a nucleoprotein of the ribose type, and its degradation and resynthesis in the chromatolytic cycle has recently been studied with the quantitative histochemical method of Caspersson[1] by Gersh and Bodian.[2] The important role of enzymatic activity in these cellular reactions suggested that demonstrable changes in phosphatase activity could be found associated with the considerable turnover of nucleoprotein, and of chemical energy, in the chromatolytic process. The development of a histochemical method for phosphatase determination by Takamatsu[3] and by Gomori,[4] and the finding of demonstrable acid phosphatase activity in the cytoplasm of normal nerve cells with this method has led us to a study of the changes in, and localization of, phosphatase activity during the chromatolytic cycle. These studies are still in progress, but our early findings may be of sufficient general interest to merit a preliminary report.

The details of our methods have been modified from those published by Wolf. Kabat, and Newman[5] for the study of acid phosphatase activity. The procedure consists essentially in the incubation for 5 to 24 hours of paraffin sections of acetone-fixed spinal cord in a buffered bath containing a phosphate ester and lead nitrate. Inorganic phosphate released by enzyme activity is precipitated *in situ* by the lead reagent, and a dark lead sulfide is subsequently formed by treating the sections with dilute ammonium sulfide. The sites of phosphatase activity thus appear as brown-to-black "stained" areas in the microscopic section. Control sections in which phosphatase activity has been inhibited by dilute sodium fluoride in the incubating mixture show very little or no staining. Other control sections give excellent staining with toluidin blue and gallocyanin and permit a quite satisfactory comparison of the degree of chromatolysis as visualized with these standard histological methods.

A high degree of consistency of staining throughout the block of spinal cord from which paraffin sections were made was obtained by vascular perfusion of the fixative (chilled acetone) through the anesthetized animal. Chromatolysis was studied in the anterior horn cells of rhesus monkeys in which the sciatic nerve on one side had previously been sectioned. Nerve cells of the sciatic nerve groups on the opposite side of the same spinal cord sections served as normal controls. By varying the time of incubation in the substrate mixture an optimum degree of "staining" could be obtained for comparison of chromatolytic and normal nerve cells.

The substrates used which gave most satisfactory results were sodium glycerophosphate, adenylic acid, a mixture of pentose nucleotides from ribonucleic acid, and nucleic acid. Wolf. Kabat, and Newman[5] have shown that phosphatase activity at pH 5.0 is present in the cytoplasm of nerve cells, whereas at pH 9.0 it is absent in this site but present in mesodermal structures such as blood vessels. Consequently, most of our experiments were carried out on incubating solutions buffered at pH 5.0. When difficulty was encountered in preparing the incubating solution at pH

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[1] Caspersson, T., *Skand. Arch. f. Physiol.*, 1936, **73** (Suppl.), 1.

[2] Gersh, I., and Bodian, D., *J. Cell. and Comp. Physiol.*, 1943, **21**, 253.

[3] Takamatsu, H., *Tr. soc. path. Jap.*, 1939, **29**, 492.

[4] Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **43**, 23.

[5] Wolf, A., Kabat, E. A., and Newman, W., *Am. J. Path.*, 1943, **19**, 423.

5.0, such as when nucleic acid was used as a substrate, the substrate mixture was buffered at pH 7.0. At this pH level phosphatase activity characteristic of both the acid and alkaline ranges occurred, with staining of neurones as well as of blood vessels.

The results obtained gave unequivocal and precise information under the conditions described above, and it is possible at the present time to summarize the changes in phosphatase activity occurring in the chromatolytic cycle from the 1st to the 46th day after nerve section. With standard histological methods the degradation of Nissl substance in our material is first clearly visible at 3 days and reaches its height at 10 days. Following this period, restitution of the Nissl material occurs progressively. This regeneration is clearly apparent at 3 weeks and is well advanced at 46 days, the longest interval which we have thus far studied with the phosphatase method. It is known that complete restoration of Nissl bodies occurs in most cells after several months, although a small number of cells may fail to recover.

A marked increase in phosphatase activity occurs in the regions of the cytoplasm of chromatolytic nerve cells where Nissl material is deficient. This increase is roughly parallel in onset with earliest histological changes and is very marked at the height of chromatolysis, precisely in the region of the cytoplasm in which Nissl bodies can no longer be seen in the control sections stained with toluidin blue. Moreover, at 24 days when restitution of Nissl bodies, and presumably increased synthesis of ribonucleotides, is well under way, a marked increase of phosphatase activity still persists in chromatolytic regions. After 46 days, when many cells show well advanced restoration of Nissl bodies, the phosphatase activity in these cells is only slightly increased above normal. At this stage, cells which have lagged behind in recovery show greater phosphatase activity, and some cells which have achieved far advanced recovery of Nissl bodies show little if any increase of phosphatase activity over normal levels. It is thus likely that the phosphatase activity in chromatolytic cells is proportional to the degree of chromatolysis, during both the period of decrease and of restitution of Nissl substance. The analysis will be continued into later stages of the restitution cycle up to the period of complete morphological recovery and functional axon regeneration.

In general, similar results were obtained with the several substrates employed. The increase of phosphatase activity obtained when nucleic acid is used is a measure also of concomitant ribonuclease activity in chromatolytic cytoplasm, since phosphatase activity alone would be incapable of releasing free phosphate from nucleic acid. No increase of phosphatase activity was found in the nuclei at any stage of chromatolysis. This serves to confirm the findings of Gersh and Bodian[2] that the nucleus plays little if any role in the replacement of Nissl substance.

Previous indicators of physiological and pathological changes in the nerve cell have been alterations of stainable Nissl substance studied by staining methods or the ultraviolet absorption method. Changes in the quantity of nucleoprotein material within the cell as determined by these methods can now be supplemented by a simple histochemical enzyme analysis, so that observed changes in phosphatase and ribonuclease activity can be correlated with changes in stainable nucleoprotein material. It is hoped that the unique opportunity of interpreting nucleoprotein turnover in terms of localizable intracellular biochemical kinetics, will contribute to the analysis of the dynamic equilibrium of nucleoprotein synthesis and of energy utilization in the cell.

Summary. Markedly increased acid phosphatase activity has been found in regions of the cytoplasm of nerve cells in which chromatolysis has been produced by axon section. This increased activity is proportional to the degree of chromatolysis, and is thus probably associated with increased nucleoprotein synthesis or degradation.

14541

The Relative Toxicity of Six Salts of Penicillin.

Henry Welch, Donald C. Grove, Ruth P. Davis, and Albert C. Hunter.
(Introduced by R. P. Herwick.)
From the Food and Drug Administration, Washington, D.C.

The hygroscopic nature of commercial penicillin sodium is a definite deterrent to efficient and economic handling of this material from a production standpoint, and for this reason alone there is a definite trend towards the development of a penicillin salt which is less hygroscopic, safe, and possessing the same therapeutic powers as those shown for the sodium salt. The calcium salt of penicillin which has been produced by a number of manufacturers is less hygroscopic and more stable than the sodium salt of this compound and at the present time is being studied to determine its therapeutic value in comparison with penicillin sodium. Other salts of penicillin (magnesium, ammonium, and barium) have been commercially prepared either for clinical trial or for use as standards, but few data have been accumulated as to their safety or efficiency as therapeutic agents.

In the present study 6 salts of penicillin have been investigated for their relative toxicity in mice. Five of these salts, sodium, ammonium, strontium, potassium, and magnesium, were prepared from a single master lot of penicillin. The sixth salt studied, calcium, was a commercial preparation. The latter salt is to be used as a reference standard and is to be distributed monthly by the Food and Drug Administration to all manufacturers of penicillin.

A pooled lot of penicillin sodium was used in the preparation of the 5 salts listed above. This pooled material was made up of the products of several manufacturers and contained as well portions of several lots of penicillin prepared in our pilot plant. The pooled material, containing approximately 6,000,000 units of penicillin, was first adsorbed on charcoal. It was then eluted with 75% acetone, following which the acetone was removed by vacuum distillation. The aqueous solution remaining was acidified and extracted with chloroform. The chloroform was then divided into 5 equal portions and shaken out with the calculated amounts of the proper carbonates or hydroxides to produce the sodium, ammonium, strontium, potassium, and magnesium salts of penicillin. As soon as preparation of the various salts had been completed, each was diluted to the same volume with distilled water, ampuled, shell frozen and dried under vacuum on a Cryochem apparatus (50 microns of mercury) for a 22-hour cycle. During the last four hours of drying all ampuls were heated to a temperature of 37°-39° C.

To conserve material, and since all salts were dried at the same time in the same volume on a single apparatus, moisture determinations were not made. A portion of each salt was assayed to determine potency in units per milligram and a second portion was assayed chemically to determine percent of cation present. The remainder of each salt of penicillin was then used to determine by intravenous injection the LD_{50} in 20-g mice. The LD_{50} in 20-g mice of the cations of solutions of the acetates of sodium, ammonium, strontium, calcium, potassium, and magnesium was also determined. From 6 to 12 mice were injected at each dose level and repeat tests were made with the same numbers of animals at the critical concentrations of all the preparations studied (Table I).

Even though 5 of the 6 penicillin preparations were produced from a single master lot of penicillin, there is a marked difference in the potencies of the various salts. The lowest potency material, penicillin strontium, was found to assay 285 units per milligram, while the penicillin magnesium, which had the highest potency, assayed 1028 units per milligram. It is extremely important when discussing the relative toxicity of a group of salts of penicillin such as those described in Table I

TABLE I.
Relative Toxicity of 6 Salts of Penicillin in Mice.

Salt of Penicillin	LD_{50} in units	LD_{50} in mg of Penicillin	Units per mg	% of cation	LD_{50} in mg of cations	Milliequivalents of cations of penicillin at LD_{50}	Acetates	LD_{50} in mg of cations	Milliequivalents of cations of acetates at LD_{50}
Na	21,000	32.0	656	7.72	2.47	0.11	Na	7.8	0.34
NH_4	6,600	19.9	332	6.96	1.38	0.08	NH_4	1.9	0.11
Sr	4,850	17.0	285	13.53	2.3	0.05	Sr	3.75	0.09
*Ca	3,700	11.9	310	8.16	0.97	0.05	Ca	1.03	0.05
Mg	7,600	7.4	1028	4.23	0.31	0.03	Mg	0.38	0.03
K	2,850	5.9	479	12.45	0.73	0.02	K	1.09	0.03

* Commercial lot; all other salts prepared from a single master lot.

Relative toxicity based on milligrams of cation at LD_{50} dose of salts of penicillin:
$Na < Sr < NH_4 < Ca < K < Mg$

Relative toxicity based on milligrams of cation at LD_{50} dose of acetates:
$Na < Sr < NH_4 < K < Ca < Mg$

to give consideration to the potencies of the individual salts. If the salts of penicillin are tabulated in the order of their toxicity based on *units* at the LD_{50} dose the order would be (increasing toxicity): Na, Mg, NH_4, Sr, Ca, and K. It is obvious that magnesium, which is known to be quite toxic on intravenous injection, is not in the proper position in this series. The apparent lower toxicity of penicillin magnesium in the series is due to its high potency (1028 units per milligram). Studies have been made[1] of another lot of penicillin magnesium (commercial preparation) which had a potency of only 70 units per milligram. The LD_{50} in this case proved to be 710 units, and this dose contained 0.325 mg of Mg^{++}. It will be noted (Table I) that the LD_{50} of Mg^{++} is 0.31 mg on the above high potency material (1028 units per mg) and 0.38 mg for the magnesium ion of magnesium acetate. Obviously, therefore, with both these high and low potency samples of penicillin magnesium the toxicity is due to the cation.

If one considers the milliequivalents of the 6 cations of the penicillin salts present at the LD_{50}, then the relative toxicity would result in the following series (increasing toxicity): Na, NH_4, Sr, Ca, Mg, and K. The standard error at the LD_{50} was found to vary from a low of ±215 units with penicillin calcium to a high of ±400 units with penicillin ammonium. When the milliequivalents are computed for the cations of the acetates, the cations align themselves in the same order as did the cations of the salts of penicillin. In the case of the acetates the standard error at the LD_{50} was found to vary from a low of ±0.02 mg with magnesium acetate to a high of ±0.30 mg with calcium acetate.

The order of relative toxicity (increasing toxicity), based on milligrams of cation at the LD_{50} dose of the salts of penicillin, is Na, Sr, NH_4, Ca, K, and Mg, while the relative toxicity based on milligrams of cation at the LD_{50} dose of the acetates is Na, Sr, NH_4, K, Ca, and Mg. If the number of milligrams of the cation at the LD_{50} of the penicillin preparations is compared with the number of milligrams of the cation at the LD_{50} of the acetates, with 4 of these salts of penicillin there is little difference in the weights of the cations at the LD_{50} doses, potassium and ammonium showing a difference of 0.36 and 0.52 mg, respectively, while the difference observed with calcium is only 0.05 mg, and that of magnesium only 0.07 mg. It would appear then that so far as these 4 penicillin preparations are concerned the toxicity is primarily due to the cation. In the case of sodium and strontium, on the other hand, the conclusion is drawn that the toxicity of the penicillin preparations made from these cations is only partially due to the cation present and that some of the toxicity must be assigned to either the penicillin itself or, perhaps more likely, to organic contaminants resulting from the process of extraction. But even with these

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[1] Welch, H., Price, C. W., Neilsen, J. K, and Hunter, A. C., in press.

2 salts of penicillin it would appear from these studies that, with more potent (purer) preparations, their toxicities also would be due primarily to the concentration of the cation present.

Summary and Conclusions. Five salts of penicillin prepared from a single master lot were investigated for their acute intravenous toxicity in mice. A sixth salt, a commercial preparation of penicillin calcium, was studied.

Based on milliequivalents of the cation used in the preparation of these salts their relative toxicity was found to be (increasing toxicity): Na, NH_4, Sr, Ca, Mg, and K. The same relative toxicity was found for the acetates of these cations.

The relative toxicity based on milligrams of the cation at the LD_{50} dose of the salts of penicillin is (increasing toxicity): Na, Sr, NH_4, Ca, K, and Mg, while the relative toxicity based on milligrams of the cation at the LD_{50} dose of the acetates is (increasing toxicity): Na, Sr, NH_4, K, Ca, and Mg.

The toxicity of salts of penicillin is primarily due to the cations used in their preparation.

14542 P

The Analgesic Effect of Morphine Alone and in Combination with Dextro-Amphetamine.*

F. R. GOETZL, D. Y. BURRILL, AND A. C. IVY.

From the Department of Physiology, Northwestern University Medical School, Chicago.

Synergism of morphine and dextro-amphetamine in the production of analgesia has been demonstrated in dog experiments.[1] Investigations of a similar nature, though with a different method, have been carried out on mice. The results of these experiments are here reported.

The pain thresholds were determined in the mice by pinching the tails, as described by Haffner[2] and Molitor and Robinson.[3] A pair of dissecting forceps was modified by the placement of a rotating wedge between the blades in such a way that the approximation of the blades was limited by the thickness of the wedge at the selected setting. The rotating wedge was calibrated to indicate successive 0.5 mm closures between the tips of the blades. The forceps were applied to pinch the base of the tail and the wedge rotated to allow increasing amounts of pressure to be applied. The value indicated on the disc, referring to the spacing between the blades when the mouse indicated pain by squeaking, was interpreted as the threshold of pain. The forceps were always applied to the same part of the tail and care was taken not to cause tissue injury.

For each experiment each animal was kept in an individual cage which allowed some movement but kept the tail in proper position for the test. The mice used were all of one strain and each animal was used only once.

At the beginning of each test, before the administration of any drug, the normal sensitivity of each animal to the stimulus described was tested 3 times at 15-minute intervals. Animals which did not give uniform responses in these preliminary tests were discarded. Then the drug or drug combination was injected subcutaneously and the tests repeated at intervals of 15 minutes for 2¼ to 6 hours to follow changes in sensitivity.

Each drug and combination of drugs was tested 4 times on groups of 12 animals each. The results are given in terms of mean values for all animals in each dosage group. The mean values are accurate because there was

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* This work was aided in part by a grant from Smith, Kline, and French laboratories.

[1] Goetzl, F. R., Burrill, D. Y., and Ivy, A. C., *Fed. Proc. Am. Soc. Exp. Biol.*, 1943, **2**, 1, 16.

[2] Haffner, E., *Deutsche med. Wchnschr.*, 1929, **251**, 731.

[3] Molitor, H., and Robinson, H., *Anesth. and Analg.*, 1938, **17**, 188.

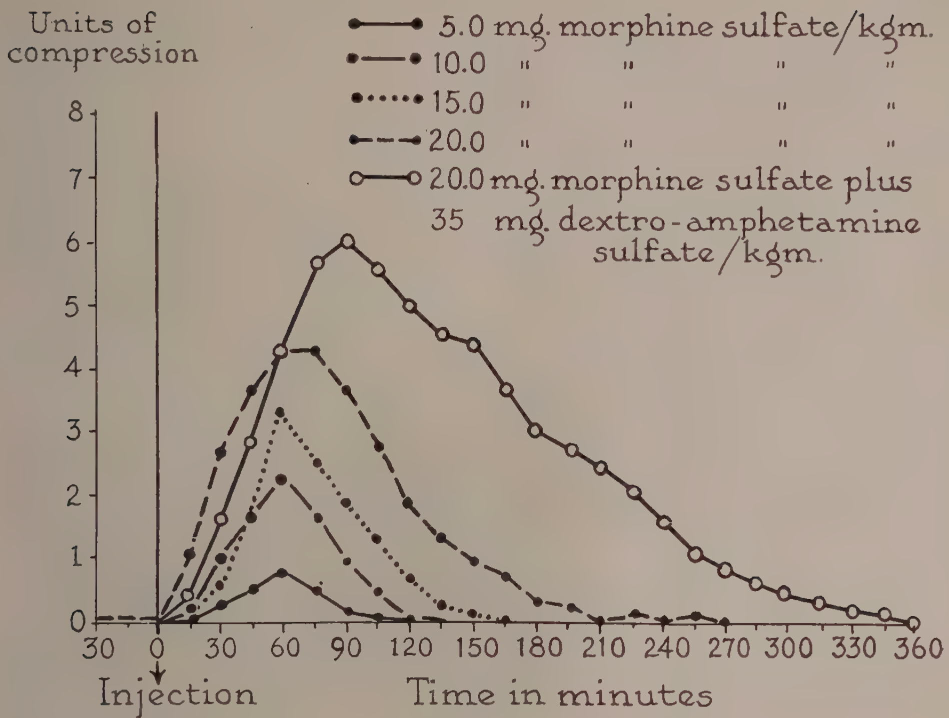

FIG. 1.

no overlapping of results in regard to maxima and durations of the effects. For instance, the least analgesic effect obtained in any animal with 20.0 mg of morphine sulfate plus 35.0 mg of dextro-amphetamine per kilogram was greater than the greatest effect obtained in any animal with 20.0 mg of morphine sulfate per kilogram alone.

In Fig. 1 the units of compression required to produce a squeak are plotted against the times at which the animals were tested. The resulting curves show the changes in sensitivity to the pain stimulus used.

The analgesic effects of 5.0, 10.0, 15.0, and 20.0 mg of morphine sulfate per kilogram are shown in the figure. The smallest doses caused only a reduction of activity in the cages, while the larger doses caused all of the animals to go to sleep. Sleeping apparently did not interfere with the tests for sensitivity, as the sensitivity returned to normal after a time, even though the animals were sleeping between tests. The 20.0 mg dose put the animals to sleep for 4 to 5 hours. After the administration of the 10.0, 15.0, and 20.0 mg doses a positive Straub reaction was seen in all animals. It will be noted from the figure that the pain sensitivity returned to normal within 3½ hours after the administration.

Changes in sensitivity produced by dextro-amphetamine alone could not be demonstrated because of the marked stimulation and increase in activity produced by even small doses. The stimulation and increased activity persisted for some time after the mice were returned to the large cages.

The analgesic effects of a combination of 20.0 mg morphine sulfate with 35.0 mg of dextro-amphetamine sulfate per kilo were readily measurable and were greater than the effects produced by the morphine sulfate alone. The changes in sensitivity are indicated in the figure. It is to be noted that the analgesia was greater and longer lasting than with 20.0 mg of morphine alone. All of the animals remained awake and alert. The Straub reaction did not develop in any animal. When they were returned to the large cages all of the mice exhibited apparently normal activity. This test continued for 6 hours, the time required for the sensitivity to return to pre-injection level.

While the analgesic effect of dextro-amphetamine alone could not be determined in this experiment it is clear that the drug increased the analgesic effect of morphine while counteracting the narcotic effect of morphine. Antagonism, except with reference to analgesia, was further shown by the inhibition of the Straub reaction.

The method used in this experiment was not entirely satisfactory, but the results obtained are in agreement with those obtained in the dog experiments.

Summary. Experiments have been made on mice which demonstrate that dextro-amphetamine increases the analgesic effects of morphine while counteracting its narcotic action. Although a different method has been used in these studies the results agree with those obtained in dog experiments.

14543

Specificity of the Epiphyseal Cartilage Test for the Pituitary Growth Hormone.*

Walter Marx, Miriam E. Simpson, and Herbert M. Evans.

From the Institute of Experimental Biology, University of California, Berkeley, California.

Recently a procedure for the bioassay of the pituitary growth hormone was published in which the width of the uncalcified portion of the proximal epiphyseal cartilage of the tibia in hypophysectomized rats was used as the index of potency.[1] In order to investigate the specificity of this test it was considered necessary to determine the reaction of the epiphyseal cartilage to other hormones. This paper reports the effects of thyroxin, and of purified pituitary thyrotropic, adrenocorticotropic, and lactogenic hormones on the cartilage under the same conditions.

The procedure was exactly as described in the paper on the standardization of growth hormone: Female rats 26 to 28 days of age were hypophysectomized and after a 12- to 13-day post-operative period were injected daily intraperitoneally for 4 days with a particular hormone. Twenty-four hours after the last injection (17 to 18 days post-operative) the rats were autopsied and the proximal end of the tibia was split and fixed in neutral formol. After staining with silver nitrate the uncalcified portion of the epiphysis was measured with microscopic low power using a micrometer eye piece.

The results are summarized in Table I. After injection of thyroxin the cartilage width was slightly greater than in the controls; though the effect was small, it did approach that produced by the growth hormone unit.[†] No gradation of effect with increasing dosage was noted.

Pituitary thyrotropic hormone had no significant effect on the epiphyseal cartilage at doses as high as 0.6 mg. The preparation was kindly furnished by J. Fraenkel-Conrat.[2] Though not devoid of contaminants the preparation was highly potent having a mini-

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[*] Aided by grants from the Board of Research of the University of California and the National Research Council Committee on Research in Endocrinology.

[1] Evans, H. M., Simpson, M. E., Marx, W., and Kibrick, E., *Endocrinology*, 1943, **32**, 13.

[†] One growth hormone unit is defined as the daily dose of growth hormone which under these conditions produces an increase of 50 micra in the width of the uncalcified proximal epiphyseal cartilage of the tibia.

[2] Fraenkel-Conrat, J., Fraenkel-Conrat, H., Simpson, M. E., and Evans, H. M., *J. Biol. Chem.*, 1940, **135**, 199.

TABLE I.

Effect of Hormones Other Than the Pituitary Growth Hormone on the Proximal Epiphyseal Cartilage of the Tibia.

Hormone	Daily dose, μg	No. of rats	Width of uncalcified cartilage: Mean, micra	Range, micra	Difference from control, micra
Thyroxin	30	15	191	138 to 248	+36
	15	39	201	146 to 286	+46
	5	31	186	120 to 287	+31
Thyrotropic hormone	600	7	184	169 to 210	+29
	200	8	157	120 to 177	+ 2
	67	7	160	143 to 186	+ 5
	22	8	139	115 to 184	—16
Adrenocorticotropic hormone	500	10	110	91 to 139	—45
	250	9	136	103 to 155	—19
	100	18	142	110 to 172	—13
	50	19	131	89 to 181	—24
	25	8	139	120 to 167	—16
Lactogenic hormone	4000	6	175	138 to 189	+20
	2000	8	179	169 to 198	+24
	1000	7	163	143 to 186	+ 8
	500	9	158	127 to 179	+ 3
Uninjected controls	0	103	155	108 to 205	0

TABLE II.

Effect of the Pituitary Growth Hormone on the Proximal Epiphyseal Cartilage of the Tibia.

Daily dose, μg	No. of rats	Width of uncalcified cartilage: Mean, micra	Range, micra	Difference from control, micra
150	15	285	220 to 309	+128
100	10	268	230 to 292	+111
50	25	249	197 to 292	+ 92
40	9	232	172 to 299	+ 75
20	19	210	151 to 308	+ 53
10	20	194	163 to 268	+ 37
5	9	175	140 to 220	+ 18
0	46	157	112 to 199	0

mum effective dose in one-day-old chicks of 20 μg.

Adrenocorticotropic hormone prepared by Li[3] in electrophoretically pure form, and containing 20 adrenal-repair units per mg, was definitely not a stimulant of the epiphyseal cartilage; in fact, the discs were consistently narrower than those of the hypophysectomized controls. A similar result has been reported for a 15-day injection period by Marx, Simpson, Li, and Evans.[4]

The epiphyseal cartilage of rats injected with lactogenic hormone prepared by Lyons,[5] and containing 25 to 30 international units per mg, was slightly wider than that of controls. The increase was not significant, however, even when the substance was injected in doses as high as 4.0 mg daily.

For purposes of comparison, the response to the hypophyseal growth hormone as regards width of the epiphyseal cartilage is shown in Table II. In these experiments, a "cysteine treated globulin fraction"[6] was used, containing approximately 25 growth hormone units per mg (body weight test in hypophysectomized rats),[7] but less than 1% of any of the other known pituitary hormones. It will be noted that in the case of the growth hormone, markedly larger effects were secured, increasing with increasing dosage. This was not the case with any of the other hormones here employed.

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[3] Li, C. H., Simpson, M. E., and Evans, H. M., *Science*, 1942, **96**, 450.

[4] Marx, W., Simpson, M. E., Li, C. H., and Evans, H. M., *Endocrinology*, 1943, **33**, 102.

[5] Lyons, W. R., *Cold Spring Harbor Symposium on Quant. Biol.*, 1937, **5**, 198.

Summary. 1. Purified anterior hypophyseal hormones other than the growth hormone were administered to hypophysectomized rats for a period of 4 days and measurements made of the width of the uncalcified portion of the proximal epiphyseal cartilage of the tibia. 2. While adrenotropic hormone slightly decreased the cartilage width, thyroxin and the thyrotropic as well as the mammotropic hormones caused slight enlargement of the epiphyseal cartilage. The effect was smaller, however, than the response caused by a single growth hormone unit, at all dose levels tested, and the effect did not show gradation with dosage which is a characteristic of the growth substance.

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[6] Marx, W., Simpson, M. E., and Evans, H. M., *J. Biol. Chem.*, 1943, **147**, 77.

[7] Marx, W., Simpson, M. E., and Evans, H. M., *Endocrinology*, 1942, **30**, 1.

14544

A Comparison of Regeneration and Respiration Rates of Tubularia.*

S. SPIEGELMAN AND A. GOLDIN. (Introduced by L. G. Barth.)

From the Department of Zoology, Washington University, St. Louis, Missouri; the Department of Biology, Queens College, New York; and the Marine Biological Laboratory, Woods Hole, Mass.

Barth[1] showed that oxygen can be made the determining factor in the polarity and rate of regeneration of Tubularia stems. In an attempt to find the metabolic basis of regeneration, Barth[2] investigated the relation between oxygen consumption rates and regeneration rates, and found a general parallelism between the two at various oxygen tensions. In the same study a comparison of the oxygen consumption rates of regenerating versus resting stems showed comparatively little difference between the two groups. This important finding indicated that while the process of regeneration depended on some portion of the aerobic metabolism, a relatively small part of the overall respiration was involved. Moog and Spiegelman[3] were able to show with the use of various respiratory poisons that complete inhibition of regeneration could be obtained without any parallel effect on respiration that was measurable.

These findings imply that the stimulatory effect on regeneration of increased oxygen tensions is a non-specific one and can be interpreted as a general influence on all oxygen consuming systems.

Goldin[4] found that at oxygen tensions at which normal regeneration rates might be expected, complete inhibition could be obtained by lowering the hydrogen ion concentration sufficiently. Therefore it was of importance to determine whether the pH effect on regeneration was mediated through its effect on the overall oxygen consumption or whether it was of a more specific nature such as was found with some of the respiratory inhibitors.

Methods. Young, unbranched Tubularia stems of uniform diameter were selected from colonies gathered from the waters of Cape Cod

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* This investigation was carried on with the aid of a Rockefeller grant administered by Dr. H. B. Steinbach.

[1] Barth, L. G., *Physiol. Zool.*, 1938, **11**, 179.

[2] Barth, L. G., *Biol. Bull.*, 1940, **78**, 366.

[3] Moog, F., and Spiegelman, S., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 392.

[4] Goldin, A., *Biol. Bull.*, 1942, **82**, 243.

Bay during the months of July and August. Stem segments, 6 mm in length, were cut from the region 5-11 mm proximal to the hydranth. The stem segments were suspended in filtered sea water, the oxygen tension and pH of which were adjusted by saturation with appropriate mixtures of oxygen, carbon dioxide, and nitrogen. The oxygen tension was kept constant at that of normal sea water, the pH being varied by means of the CO_2-nitrogen ratios.

Oxygen consumption values were obtained with Warburg manometers and vessels. All experiments were run at 19 ± 0.1°C. The manometers were shaken at a rate of 90 oscillations per minute through a 5 cm arc. Each vessel was flushed with the gas mixture used to saturate the sea water in it. The bicarbonate buffer system required the use of the indirect method of Warburg.[5] In this method 2 manometers are needed for each Q_{O_2} determination. The stems were suspended in 8 cc of sea water in one vessel and 3 cc in the other of a pair.

Wet weight determinations were made at the end of the run by means of a micro-balance after removal of excess moisture from the stem of the filter paper. Calculations are based on cu mm of O_2 consumed per mg of wet weight per hour. Hydrogen ion concentrations were determined with a glass electrode.

Results. Table I summarizes results of experiments covering a range of pH from 8.0 to 6.6. This pH range was found by Goldin[6] to include all degrees from no effect on regeneration, to complete inhibition. A determination of regeneration rates on the stems in the Warburg vessels could not be made since this would have involved opening the vessels and removing the stems for measurement during a respiratory experiment. Table II, however, gives some representative data of the variation of regeneration rate with pH. Regeneration rate is calculated as per cent of normal at pH 8.1. The data were collected in the usual fashion (Goldin, *loc. cit.*) at the same time that the respiration experiments were made, so that a comparison is possible.

TABLE I.

Variation of Respiration Rates with pH.

Rate of O_2 consumption in cmm/wet weight/hour of controls (pH 8.0) was 5.3 in Exp. 1, and 7.3 in Exp. 2. Stems were examined at end of run for signs of regeneration. + signifies regenerative activity; — signifies complete absence.

	pH	% normal respiration	Regeneration
Exp. 1	8.0	100	+
	6.8	55	—
	6.6	27	—
” 2	8.0	100	+
	7.4	115	+
	6.7	46	—
	6.6	37	—

TABLE II.

Variation of Regeneration Rates with pH.

Each value is the average of measurements on 30 stems. The t (hours) is the time to constriction. L is length of primordium at constriction.

pH	L in micra	L/t	$1/t$	% normal L/t
8.07	1054	31.7	0.302	100
7.07	848	18.3	0.216	58
6.82	699	9.3	0.141	31
6.63	29	0.3	0.110	1

Discussion. Table I shows that lowering the pH of the sea water medium is accompanied by a decrease in the respiratory rate. A comparison with the data in Table II reveals that within the same pH range a fall also occurs in the rate of regeneration.

It is evident that a parallelism, which is stronger at the lower pH values, exists between the effect of varied hydrogen ion concentrations on the two processes. Since a direct relation exists between respiration and regeneration (Barth, *loc. cit.*) it must be concluded that the greater part of the pH effect on regeneration is mediated through its effect on the rate of respiration.

It is probably that we have here, as in the case of cyanide (Moog and Spiegelman, *loc. cit.*) a non-specific inhibition of oxidative systems with a consequent depression of all activities dependent on them, among which is included the process of regeneration.

Summary. Lowering the hydrogen ion concentration produces a parallel depression of respiration and regeneration rates. It is concluded that the effect on regeneration is a non-specific one mediated through its effect on the enzyme systems involved in aerobic respiration.

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[5] Warburg, O., *Biochem. Z.*, 1924, **152**, 51.

[6] Goldin, A., *Biol. Bull.*, 1942, **82**, 340.

14545

Effect of Dietary Protein on Liver Riboflavin Levels and on Inactivation of Estradiol by Liver.*

K. Unna, H. O. Singher, C. J. Kensler, H. C. Taylor, Jr., and C. P. Rhoads.

From the Merck Institute for Therapeutic Research, Rahway, N.J., and the Memorial Hospital for Treatment of Cancer and Allied Diseases, New York City.

In the course of a study on the effect of vitamin deficiencies on the metabolism of estrogenic hormones,[1] it was found that the livers of thiamine- as well as of riboflavin-deficient rats failed to inactivate estradiol whereas the liver of animals depleted in pyridoxine, pantothenic acid, biotin, or vitamin A retained the ability to metabolize the hormone. Furthermore, determinations of thiamine and riboflavin levels in the liver of deficient animals demonstrated a direct relationship between the vitamin content of this organ and its ability to inactivate estradiol. In continuation of these studies the influence of the protein intake upon the riboflavin content of the liver and the ability of liver tissue to inactivate estradiol was investigated.

Weanling rats of both sexes were placed on a basal diet containing casein as the only source of protein. The diet had the following composition: casein, vitamin free, 8%; dextrose, 78%; salt mixture, U.S.P. XI No. 1, 4%; hydrogenated vegetable oil (Crisco), 8%; and cod liver oil, 2%. Several groups of animals maintained on this low protein diet received a daily supplement of 25 mg of *dl*-methionine or of 25 mg *l*-cystine. An additional group of rats was placed on an adequate protein intake by increasing the casein content of the basal diet to 18%, compensating for the change by a corresponding decrease in dextrose. The diets were fed *ad libitum*. All animals received by stomach tube a daily supplement of B vitamins consisting of 40 γ each of thiamine and pyridoxine; 80 γ of riboflavin; 200 γ of calcium pantothenate; 500 γ of nicotinamide and 5 mg of choline.

Animals of the various groups were sacrificed at intervals simultaneously with litter mates from the control group maintained on 18% casein. The riboflavin content of the liver was determined by the method of Hodson and Norris[2] and expressed in γ per gram fresh tissue. The inactivation of estradiol by 100 mg of liver slices was tested by an assay method based on increase in weight of the immature rat uterus.[3]

Results obtained on a total of 59 rats are summarized in Table I. The low protein diet retarded markedly the growth of the animals; the addition of methionine allowed for a moderate increase in growth. The growth promoting effect of 25 mg *l*-cystine under these conditions was about the same as that of 25 mg *dl*-methionine.

Whereas the riboflavin concentration in the liver of the rats maintained on 18% casein remained practically unchanged throughout the period of 3 months, a gradual decline was observed in rats maintained on 8% casein; after 3 months the riboflavin concentration in their livers was almost half of that of the control animals. The supplementation with methionine tended to offset the decrease in riboflavin. On the other hand, the animals receiving cystine, in spite of their somewhat superior growth rate, showed, after 3 months, riboflavin values in the same low range as those without any supplement.

All rats maintained on the low protein diet for a period of 3 months failed to inactivate estradiol. Supplementation with methionine enabled these animals to maintain their ability to inactivate the hormone, whereas with

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* This work was carried out with the aid of a grant from the Nutrition Foundation.

[1] Singher, H. O., Kensler, C. J., Taylor, H. C., Jr., Rhoads, C. P., and Unna, K., *J. Biol. Chem.*, in press.

[2] Hodson, A. Z., and Norris, L. C., *J. Biol. Chem.*, 1939, **131**, 621.

[3] Lauson, H. D., Heller, C. G., Golden, J. B., and Sevringhaus, E. L., *Endocrinology*, 1939, **24**, 35.

TABLE I.
Effect of Dietary Protein on Concentration of Riboflavin in the Liver and the Ability of Liver Tissue to Inactivate Estradiol.

Dietary protein	Days on diet	No. of animals	Body wt, g		Riboflavin, γ/g		Estradiol	
			Avg	Range	Avg	Stand. error	No. of tests	Inactivation
18% casein	37-57	6	178	126-227	23.6	1.3	4	4+
8% ”	37-57	9	72	57-87	17.1	1.8	6	6+
8% ” 25 mg methionine daily	37-57	9	92	74-106	18.6	1.5	6	5+; 1±
18% Casein	89-98	8	281	218-330	22.5	0.8	8	8+
8% ”	89-98	10	114	97-137	12.5	0.8	9	9—
8% ” 25 mg methionine daily	89-98	12	136	101-165	15.6	0.9	11	11+
8% ” 25 mg cystine daily	98	5	141	125-186	12.9	1.4	5	2+ ;3—

cystine, only 2 out of 5 animals were able to inactivate estradiol.

Discussion. Evidence of an interrelationship between protein metabolism and certain vitamins of the B-complex has recently been obtained. Riboflavin deficiency in chicks decreases the utilization of protein.[4] Sarett *et al.*[5] reported that the urinary excretion of riboflavin in rats bears an inverse relationship to the level of protein intake. Later, Sarett *et al.*[6] found that the concentration of riboflavin in the liver varies directly with the level of protein in the diet and is independent of the vitamin intake. Our results confirming those of Sarett likewise demonstrate a relationship between dietary protein intake and retention of riboflavin in the liver. In spite of the ingestion of liberal amounts of the vitamin the livers of rats maintained on a low protein diet are unable to retain riboflavin. Our data indicate that methionine plays a role in correcting this defect.

Prolonged protein deficiency, which is known to damage the liver in a variety of functions, was found to produce failure in the inactivation of estradiol by liver tissue. This dysfunction can be prevented by the feeding of methionine. The concomitant decrease in riboflavin concentration of the liver alone can not explain these findings. Three animals of the group maintained on 8% casein for 3 months had riboflavin concentrations above 14 γ per gram (14.3, 16, and 17 γ respectively), and 3 animals of the group receiving methionine in addition showed values below 14 γ per gram (11, 11.7, and 12.5 γ respectively). The livers of the rats in the first group failed to inactivate estradiol, whereas these supplemented with methionine did inactivate the hormone. On the other hand, in a previous study on a large number of riboflavin-deficient rats maintained on 18% casein, a concentration of 14 γ riboflavin per gram liver was found to be a critical level; none of the livers with less than 14 γ riboflavin per gram inactivated estradiol, and all livers with a riboflavin concentration above 14 γ per gram invariably inactivated the hormone.[1]

Therefore, the function of liver tissue in inactivating estradiol appears to depend on an adequate dietary intake, not only of thiamine and of riboflavin,[1] but also of protein, in which the sulfur-containing amino acids may be essential. In this respect our findings bear an analogy to the protective influence of dietary ingredients against the tumor producing effects of butter yellow. The pathologic changes in the liver following the ingestion of butter yellow in animals maintained on diets inadequate both in vitamins and in protein could be prevented to a large extent by the feeding of riboflavin and casein.[7] The effect of riboflavin *per se* could be demonstrated on animals maintained on adequate levels of protein[8] and that of casein and especially of the amino acids methionine and cystine on rats receiving adequate amounts of vitamins.[9]

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[4] Kleiber, M., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 34.

[5] Sarett, H. P., Klein, J. R., and Perlzweig, W. A., *J. Nutr.*, 1942, **24**, 295.

[6] Sarett, H. P., and Perlzweig, W. A., *J. Nutr.*, 1943, **25**, 173.

[7] Kensler, C. J., Sugiura, K., Young, N. F., Halter, C. R., and Rhoads, C. P., *Science*, 1941, **93**, 308.

Summary. Rats maintained on a low protein diet were unable to retain normal amounts of riboflavin in the liver. Methionine tended to counteract the decrease in the riboflavin concentration of the liver. The livers of rats maintained on a low protein diet for a period of 3 months failed to inactivate estradiol. Supplementation with methionine maintained the ability to inactivate estradiol.

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[8] Antopol, W., and Unna, K., *Cancer Research*, 1942, **2**, 694.

[9] György, P., Poling, E. C., and Goldblatt, H., Proc. Soc. Exp. Biol. and Med., 1941, **47**, 41.

14546

Acetylcholine Content of the Myenteric Plexus and Resistance to Anoxia.

John H. Welsh and Jane E. Hyde. (Introduced by F. L. Hisaw).
From the Biological Laboratories, Harvard University.

It has been pointed out in an earlier paper[1] that the order of increasing resistance of the parts of the mammalian nervous system to anoxia and hypoglycemia is essentially the same as the order of parts arranged according to increasing amounts of acetylcholine (ACh) per unit weight. Those parts which are least resistant (cerebellum and cortex) are low in ACh and those which are among the more resistant (spinal nerves and autonomic ganglia) are high in ACh.

Cannon and Burket[2] have shown the cells of the myenteric plexus to be highly resistant to oxygen lack, enduring 3 hours of complete anemia. Van Liere[3] has found the alimentary canal to be affected significantly only by a strong degree of anoxia. It is of interest, therefore, to know the normal level of ACh in the myenteric plexus. Dikshit[4] has shown that the rate of formation of ACh by pieces of intestine *in vitro* is as high as that of equivalent amounts of nervous tissue but, so far as we are aware, no one has attempted to estimate the ACh-content of the enteric plexuses. This we have done for Auerbach's plexus in the rabbit and guinea pig and it will be seen that, in these species, the ACh-content of this plexus is extraordinarily high.

Methods. Tissue for assay was obtained by stripping off the longitudinal muscle and serosa of the entire small intestine of adult guinea pigs and rabbits. Most of Auerbach's plexus adheres to the longitudinal muscle. Assays were also made on pieces of whole intestine. The tissues were rinsed, carefully blotted to remove excess moisture, and weighed. They were ground, with a small amount of a phosphate medium plus eserine 1:5,000, acidified to pH 3-4 with normal HCl, and allowed to stand for one hour or more. They were then neutralized with normal NaOH and the volume was adjusted to give 100 mg tissue/cc fluid. After centrifuging, the supernatant fluid was further diluted for assay. This method gives the "total" ACh. Assays were made using the ventricle preparation ("heart") from Venus mercenaria, or the frog's rectus abdominis muscle. An estimate of the relative proportion of nervous to nonnervous tissue in the removed outer layers was obtained by projecting a stained preparation of the myenteric plexus of the rabbit and making a tracing. Cutting out and weighing the parts gave an estimate of the area of the plexus and the thickness was obtained from cross sections. This method indicated that

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[1] Welsh, J. H., and Hyde, J. E., *J. Neurophysiol.*, 1944, **7**, 41.

[2] Cannon, W. B., and Burket, I. R., *Am. J. Physiol.*, 1913, **32**, 347.

[3] Van Liere, E. J., *Anoxia*, Univ. Chicago Press, 1942.

[4] Dikshit, B. B., *Quart. J. Exp. Physiol.*, 1938, **28**, 243.

TABLE I.
Acetylcholine Content of Small Intestine and Outer Layers (Myenteric Plexus, Longitudinal Muscle and Serosa) of Rabbit and Guinea Pig.
(Figures in parentheses represent values obtained from assays using frog's rectus, those not in parentheses from assays using the Venus' heart.)

	Whole intestine, γ ACh/g		Outer layers, γ ACh/g	
Rabbit No. 1	—		16	
,, ,, 2	3.3		10	(15)
,, ,, 3	4.0	(4.0)		(10)
Guinea pig 1	—		20	
,, ,, 2	6.5		16	
,, ,, 3		(8)		(20)
,, ,, 4		(10)		(20)
,, ,, 5	4.0		—	
,, ,, 6	7.5		—	
,, ,, 7	8.6		—	

the plexus constitutes roughly 1/40 of the total mass of nerve, muscle, and serous tissues.

Results. The results are given in the accompanying table. The values for total ACh in the small intestine of the rabbit (3.3 and 4 γ/g) correspond closely with those obtained by Chang and Gaddum[5] (2.8 and 4 γ AChCl/g). The extracts of the longitudinal muscle and serosa with the attached plexus gave values for the rabbit ranging from 10 to 16 γ/g. These latter values must be multiplied by 40 to obtain an estimate of the ACh per gram of Auerbach's plexus. This is assuming that all of the ACh is in the plexus, for which there is good evidence.[4]

Intestinal tissue of the guinea pig yielded values ranging from 4 to 10 γ ACh/g (avg 7.4 γ); while the removed outer layers, with the attached plexus, gave values of 16 and 20 γ ACh/g. Dikshit[4] found that guinea pig intestine synthesized ACh at a rate twice that of rabbit intestine. This difference in rate corresponds with the difference in ACh content of the intestines of the two species (rabbit, avg 3.8 γ/g; guinea pig, avg. 7.4 γ/g). No attempt was made to estimate the relative weight or mass of the myenteric plexuses in the guinea pig and rabbit, hence it is not known whether the higher values for the former are due to a proportionately greater amount of nerve tissue or a higher level of ACh in a given amount of tissue.

Discussion. To obtain an estimate of the ACh per gram of Auerbach's plexus of the rabbit one must multiply the values obtained for extracts of the outer layers by 40. This yields values ranging from 400 to 640 γ/g of plexus. Assuming the proportions of ganglionic to muscle and serous tissues to be the same in guinea pig and rabbit, corresponding values for Auerbach's plexus in the guinea pig run as high as 800 γ/g. The highest values obtained previously for parts of the mammalian nervous system are those for autonomic ganglia. MacIntosh[6] found values ranging from 12-44 γ/g in autonomic ganglia of the cat. Feldberg[7] obtained values for the superior cervical ganglia of the cat ranging from 23.5 to 36.8 γ/g.

The gray matter of the cerebrum of the cat has an ACh content of 1.2-4.5 γ/g[6] and fails to recover after periods of complete arrest of body circulation of 3-7 minutes.[8] Superior cervical ganglia of the cat have an ACh content about 10 times that of cortical gray matter and survive oxygen lack for 60-70 minutes.[9] Calculated from the rate of ACh synthesis, the myenteric plexus of the cat appears to have about the same ACh content as the rabbit[4] (roughly 10 times that of cervical ganglia) and survives complete anoxia for 3 hours.[2] Thus further evidence is provided indicating a correlation between ACh content and resistance to anoxia in parts of the nervous system.

Summary. It is shown that the estimated ACh-content of the myenteric plexus of the rabbit and guinea pig (400-800 γ/g) is higher than that of any other mammalian nervous tissue. Since the cells of this plexus are known to be the most resistant of all nerve cells to anoxia, further evidence is provided indicating a relationship between resistance to oxygen-lack and ACh-content of parts of the nervous system.

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[5] Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

[6] MacIntosh, F. C., *J. Physiol.*, 1941, **99**, 436.

[7] Feldberg, W., *J. Physiol.*, 1943, **101**, 432.

[8] Weinberger, L. M., Gibbon, M. H., and Gibbon, J. H., Jr., *Arch. Neurol. and Psychiat.*, 1940, **43**, 961.

[9] Bargeton, D., *Am. J. Physiol.*, 1938, **121**, 261.

14547 P

Integration of Cutaneous and Auditory Sensibilities in the Inferior Colliculus.

BERRY CAMPBELL. (Introduced by A. T. Rasmussen.)

From the Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minn.

The intimate relation of the spino-thalamic tract to the inferior colliculus has been shown by Rasmussen and Peyton.[1] The following study was an attempt to determine whether a special integrative function was subserved by the long spinal fibers which enter the inferior colliculus. This was carried out with micro-electrodes and the cathode-ray oscillograph. Cats and rats were used as experimental animals, Nembutal the anesthetic. For auditory stimulation, single condenser discharges were led through a piezo crystal. The nerves were stimulated by means of condenser discharge. The micro-electrodes were made by grinding sewing needles down to fine caliber and insulating them with enamel.

Contralateral clicks produce a potential in

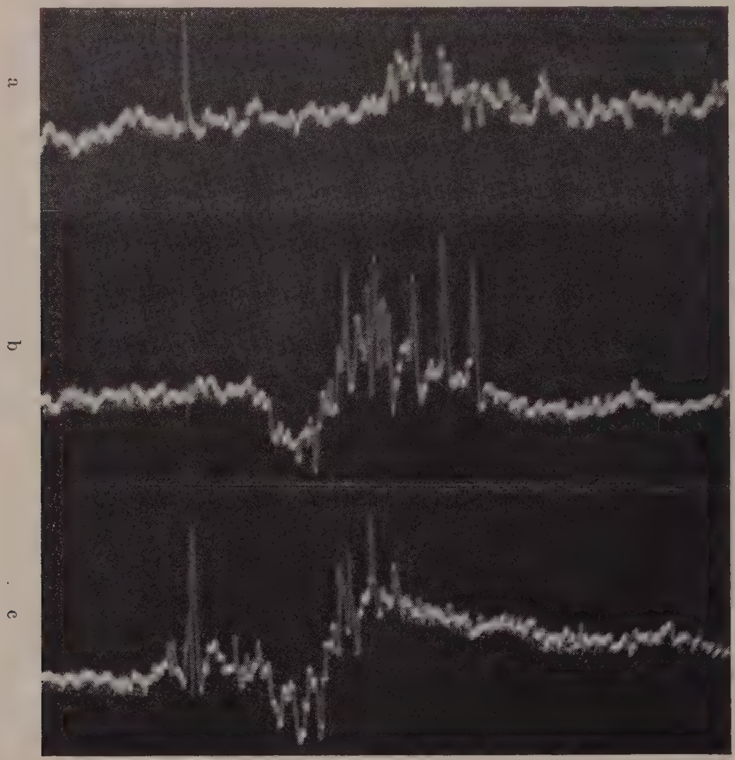

FIG. 1.

Micro-electrode recordings from inferior colliculus of cat (5-1-42). (a) response to stimulation of contralateral saphenous nerve. (b) To click in opposite ear. (c) To click in opposite ear which has been preceded by a shock to the saphenous nerve. The shock artefact of the conditioning stimulus is off of the record to the left. Time in milliseconds.

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[1] Rasmussen, A. T., and Peyton, W. T., *Surg.*, 1941, **10**, 699.

the inferior colliculus which, recorded with a single active micro-electrode, show a slow positive deflection followed by a longer negative one. Spikes, indicating the activity of single cells near the tip of the micro-electrode appear as the potential goes from positive to negative. The overall length of the potential is about 20 milliseconds.

The inferior colliculus will also respond to stimulation of contralateral cutaneous nerves. Those tested were the great auricular, median, ulnar, saphenous, tibial. The nerve to the semimembranosus failed to evoke a response in this region. Apparently the whole nucleus of the inferior colliculus was involved in these responses, but the records were easier to secure and showed more extensive firing of cells in the lateral portion of the nucleus. Instead of showing the characteristic positive-negative succession of deflections, the potentials evoked by stimulation of spinal nerves usually were characterized by a spikiness of the line, all discrete spikes being (as in the auditory potentials) negative. (Fig. 1, b). The earliest activity recorded following stimulation of the forelimb nerves in the cat was observed at 4 milliseconds, and following hindlimb stimulation 8 milliseconds.

When the spinal nerve stimulation was used to condition the auditory potential by placing it about 12 milliseconds ahead of the latter, 2 effects were noted. The first was a decrease in the duration of the spiking component of the auditory potential. This is due to the shortening of the train of spikes in the individual units (Fig. 1, c). The second effect of the conditioning is the decrease in the number of units involved in the auditory potential. Detailed analyses of the potentials of the inferior colliculus will be published elsewhere.

Summary. Stimulation of cutaneous nerves and of the cochlea evokes characteristic potentials in the inferior colliculus. With micro-electrode recording, the activity of individual cells may be observed. Placing the cutaneous stimulation before the auditory stimulation demonstrates a conditioning of the auditory potential which decreases the number of cells fired and shortens the train of impulses from those cells left active.

14548

Ultraviolet Absorption Spectrum of Human Seminal Plasma.*

Victor Ross and Lucille Ross.

From the Department of Biochemistry, College of Physicians and Surgeons, Columbia University.

Although ultraviolet absorption spectra of several body fluids have been published, we have found none of seminal plasma. Accordingly, during the course of electrophoretic and chemical examination of human seminal plasma, the following measurements of such absorption spectra were made and some factors affecting these spectra were studied.

Methods and Observations. The spectra were measured with a Hilger quartz spectroscope No. E-316 equipped with a Spekker photometer and with a hydrogen discharge tube designed by Darby.[1] Details of curve structure were not sought, principal interest being in the positions of maxima and minima and in shifts resulting from changes in pH values of the solutions.

Semen was centrifuged and the plasma dialyzed at 3°-5° in viscose casing against 0.02 M phosphate buffer containing 0.055 M NaCl (pH 7.85) and diluted for spectroscopic

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* This work was made possible by a grant from the Committee on Maternal Health, Inc., whose aid is gratefully acknowledged.

[1] Darby, Hugh H., *J. Am. Chem. Soc.*, 1940, **62**, 1874.

[2] Ross, V., Moore, D. H., and Miller, E. G., Jr., *J. Biol. Chem.*, 1942, **144**, 667.

examination. Electrophoretic patterns,[2,3] following such treatment, show a slow moving component P1 ($\mu = -0.6$)[†] representing proteose, a considerable portion of which is lost in dialysis. There are 3 globulins,[‡] P2 ($\mu = -2.9$), P2a ($\mu =$ *ca.* -3.8), and P3 ($\mu = -4.6$). In addition, 2 faster moving peaks are seen, P4 ($\mu = -5.7$) and P5 ($\mu = -6.3$), though not frequently together in the same pattern. These represent the glycoproteins. Serological tests indicate that a little serum albumin is present also, but that P2, P2a and P3 are not identical with serum globulins.

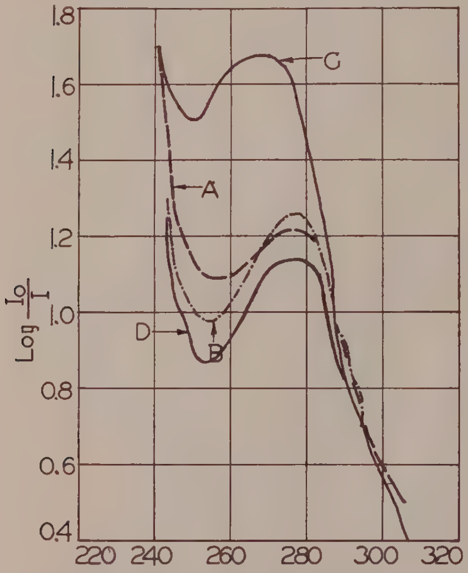

FIG. 1.

Curves A and B, normal seminal plasma. Curve C, normal seminal plasma not dialyzed. Curve D, mixture of globulins P2 and P3. Nitrogen content of solutions: A = 0.0109%; B = 0.0208%; C = 0.0102%; D = 0.0155%. All were done at pH 7.85 in a 1 cm cell. Electrophoretic analyses of plasmas corresponding to Curves A and B appear as Patterns 1 and 3 in Reference 3.

Curves A and B in Fig. 1 represent the spectra for 2 normal plasmas dialyzed as described. The maxima are at 276 and 277 mμ, the minima at 256 and 254 mμ respectively. Curve C in Fig. 1 shows absorption by an *undialyzed*, normal specimen which was simply diluted with phosphate buffer for examination. The maximum is at 268 mμ and the minimum at 250 mμ. The shift toward the more characteristic positions for proteins, following dialysis, may be due to the removal of dialyzable constituents.[§]

An absorption curve for a mixture of the globulins P2 and P3, in phosphate, is shown in Curve D, Fig. 1. The maximum and minimum are at 278 mμ and 253 mμ respectively. The globulins, therefore, determine the general shape of the curve for the whole plasma just as the proteins of blood serum determine the shape of the absorption curve of that fluid.[4]

Resembling the absorption by normal plasmas was that (not illustrated) by dialyzed seminal plasma from 3 "abnormal" semens: (a) in which the sperm were non-motile and included many morphologically abnormal forms; (b) in which repeated specimens from the same individual had cells of poor motility; and (c) in which both numbers and motility were poor. The absorption densities, log (I_o/I) of normal and abnormal specimens, were not proportional to their nitrogen content, perhaps owing to the presence of different amounts of glycoprotein.

The influence of marked changes in hydrogen ion concentration on absorption by a normal, dialyzed seminal plasma is shown in curves A (pH 7.85) and B (pH 12), Fig. 2. At pH 12 the peak has shifted to 283 mμ and the valley to 272 mμ, while the ratio of

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[3] Ross, V., Miller, E. G., Jr., Moore, D. H., and Sikorski, H., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 179.

[†] The symbols P1, P2, P2a, P3, P4, and P5 have been used in an earlier publication[3] for the protein components of human seminal plasma in the order of increasing mobility. Electrophoretic mobilities, as given in the text, are in cm^2 $volt^{-1}$ $sec.^{-1} \times 10^{-5}$ and are averages of a number of determinations.

[‡] We refer to components P2, P2a, and P3 as globulins because there are water-soluble and water-insoluble fractions of each in *fresh* seminal plasma.

[§] Some of the additional substances present in seminal plasma, and presumably removed by dialysis, are urea, glucose, lactic acid, ascorbic acid, spermine, bicarbonate, phosphate, chloride, and citrate.

[4] Lewis, S. Judd, *Proc. Roy. Soc. Lond.*, 1915-17, **89**, 327; Stenstrom, W., and Reinhard, M., *J. Biol. Chem.*, 1925, **66**, 819.

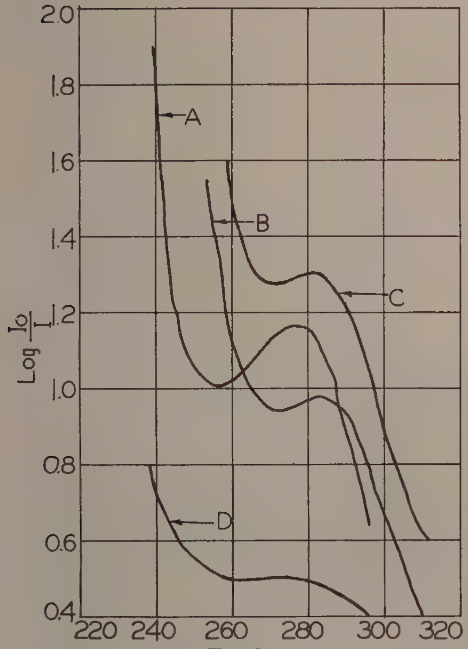

FIG. 2.

Normal seminal plasma at (A) pH 7.85 and (B) pH 12. Electrophoretic analysis of this plasma appears as Pattern 2 in Reference 3. Mixture of globulins P2, P2a and P3 at (C) pH 12 and (D) pH 2. Nitrogen content in (A) and (B) = 0.0101%; in (C) = 0.0028%; in (D) = 0.0021%. 1 cm cell used in (A) and (B), 4 cm in (C) and 2 cm in (D).

the density at the peak to that at the valley has decreased. At pH 2 the peak and valley disappeared, the curve became almost horizontal between 250 mμ and 270 mμ and the densities increased. Because the solution was somewhat cloudy and consequent scattering of light introduced errors, the curve is not reproduced. The effect of pH on the absorption by a mixture of the globulins P2, P2a, and P3 appears in curves C and D, Fig. 2. Here the shift, at pH 12 (C), was to 283 mμ for the maximum and to 272 mμ for the minimum, values identical with those for the plasma after adjustment to this pH. A similar, but more marked shift occurs with solutions of tyrosine,[5] and to this is ascribed the behavior, under these conditions, of proteins containing this amino acid. At pH 2 (D) the peak and valley disappeared but no significant change in densities occurred. At pH 7.85 the curve was almost the same as A.

Curves A and B, Fig. 3, show absorption by two preparations of P5, while curve C represents absorption by P4; there is little or no selective absorption by these glycoproteins. All 3 preparations were electrophoretically homogeneous at pH 7.85. The similarity in the curves for P5 and P4 is paralleled by the similarity in their nitrogen content and in their content of reducing substance after hydrolysis. At pH 12 (Curve D), the intensity of absorption by P4 is somewhat depressed and there is, perhaps, a slight shift toward the longer wave lengths of the small inflection in the region of 285 mμ. At pH 2 the solution became cloudy and the curve, which showed no significant change in shape but a 5-fold increase in density values, is therefore not reproduced. There is no spectrographic evidence for the presence of nucleoprotein in either of the two acetic acid precipitable components.

Summary. At pH 7.85 the peak of ultraviolet absorption by human seminal plasma and of a mixture of globulins derived from it lies in the region of 276 to 279 mμ and the valley lies between 254 and 256 mμ. No significant differences were observed between the curves for plasmas from normal semen and the curves for plasmas from 3 semens which contained sperm considered to be defective in motility or morphology. Absorption by 2 acetic acid precipitable components (glycoprotein) shows little selectivity; there is only a slight inflection in the curve in the region of 285 mμ. The absorption increases sharply in the lower wave lengths. The effect of increasing the pH of the solution to 12 is to shift absorption, by both seminal plasma and by the globulins, toward somewhat longer wave lengths. At

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[5] Holiday, E. R., *Biochem. J.*, 1936, **30**, 1795; Dhéré, C., *Recherches Spectrographiques sur l'absorption des Rayons ultraviolets par les albuminoïdes, les protéides et leures dérivés*, Fribourg, 1909; Kober, P. A., *J. Biol. Chem.*, 1915, **22**, 433; Gróh, J., and Hanák, M., *Z. Physiol. Chem.*, 1930, **190**, 169; DeGouveia, A-J. A., Coelho, F. P., and Schön, K., *Fevista da Faculdade de Ciencias da Universidada de Coimbra*, 1938, **6**, No. 4.

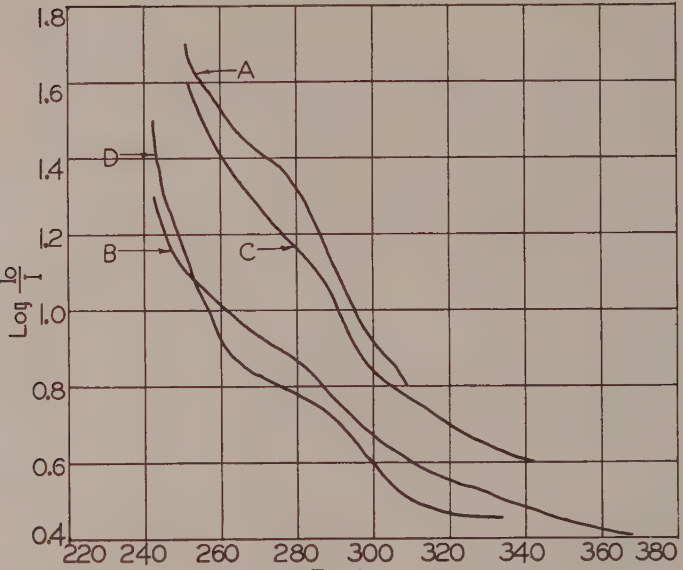

FIG. 3.

Curves A and B, two preparations of P5 (glycoprotein) examined at pH 7.85. Nitrogen content in A = 0.0022%; cell length 1 cm. Curves C and D, preparation of P4 (glycoprotein) examined at pH 7.85 and 12 respectively. Nitrogen content = 0.00136% in both; cell length 2 cm.

pH 12 there is a shift toward the longer wave lengths of the slight inflection at 285 mμ in the curve for glycoprotein.

We wish to record our appreciation to Dr. Hugh H. Darby for a discussion of the contents of this report.

14549 P

Studies on Synergism of Leukemogenic Agents in Mice.*

HENRY S. KAPLAN† AND ARTHUR KIRSCHBAUM.

From the Departments of Radiology and Anatomy, University of Minnesota Medical School, Minneapolis, Minn.

Various agents have been shown to act synergistically with carcinogens when applied simultaneously to susceptible mice.[1,2] Furth and Boon[3] recently reported a synergistic leukemogenic effect of combined application of methylcholanthrene and irradiation in Rf/Ak hybrids. The incidence of leukemia was 6% in irradiated mice receiving a single dose of 175 r, 16% in others treated only with the carcinogen, and 64% in irradiated and carcinogen-treated mice. The onset of leukemia was also accelerated in animals receiving combined treatment. Preliminary work revealed a 59% incidence of leukemia in animals

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* This investigation has been aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research and the Cancer Fund of the Graduate School of the University of Minnesota.

† National Cancer Institute Trainee in Radiology.

[1] Berenblum, I., *Cancer Research*, 1941, **1**, 807.

[2] Shear, M. J., *Am. J. Cancer*, 1938, **33**, 499.

[3] Furth, J., and Boon, M. C., *Science*, 1943, **98**, 138.

treated with X-rays alone using a single dose of 300 r.

Strain Dba[‡] and strain F mice were used in the present experiments to determine whether the findings of Furth and Boon are applicable to other mice known to develop leukemia. Strain Dba exhibits marked susceptibility to the induction of leukemia by chemical carcinogens,[4,5,6] and strain F mice have a high incidence of spontaneous leukemia,[7] the onset of which can be accelerated by treatment with methylcholanthrene or benzpyrene.[8,9] Except for the genetically different stocks of mice employed, the investigations being reported paralleled those of Furth and Boon. However, fractionated dosage technic of irradiation was used, and higher total doses were administered in an attempt to accentuate the effect of the physical agent. The irradiated animals received a daily dose of 80 r, using 140 K.V., 30 cm skin target distance, 2 mm Al filter, including the entire body in the field. Nine successive daily treatments were administered to the strain F mice for a total of 720 r, while the more hardy strain Dba animals received a total dose of 880 r. Three experimental groups were established. In one, irradiation alone was used; in a second, 0.5% methylcholanthrene was applied percutaneously twice weekly; and a third received both types of treatment, the first application of carcinogen being given on the first day of irradiation

Four deaths occurred in the group of strain Dba mice receiving combined treatment, and 14 of the surviving 24 animals, or 58%, developed leukemia at an average of 127 days after the inception of treatment. Thirty-eight, or 60%, of 63 non-irradiated, carcinogen-treated strain Dba mice developed leukemia at an average of 114 days after the first percutaneous application. Only one post-irradiation death occurred in the group subjected to X-rays alone; all of the remaining 22 animals are in excellent condition and none have developed leukemia 5 months after irradiation. However, since the leukemogenic effect of irradiation alone was reported by Furth and Boon to require a considerably longer period to become manifest, the results in this group must be regarded as preliminary and subject to revision.

Although a smaller dose of X-rays was administered to the strain F mice, it proved lethal to almost one-half of the mice which were also treated with methylcholanthrene. Of 21 mice surviving 100 days or more after combined treatment, only 2 have developed leukemia, whereas 12 of 39 non-irradiated mice treated only with the carcinogen have died of leukemia (190 days have elapsed since beginning of experiment), with 20 animals still alive. Inasmuch as the effect of combined X-ray and methylcholanthrene treatment proved lethal to such a large proportion of mice, these results may be open to question. The experiments on strain F mice are therefore being repeated using a single small dose of X-rays. Only 4 of 38 strain F mice receiving irradiation alone died of the treatment, and none of the survivors have developed leukemia (5 months after the first treatment).

Contrary to the experience of Furth and Boon, no synergistic effect of irradiation, when used in combination with a chemical carcinogen, was noted on the induction of leukemia. The only non-genetic difference between the otherwise similar experiments lies in the higher dosage and somewhat different method of irradiation. The importance of the dosage factor is now being tested. The difference in genetic constitution might well account for the antithetical results of the present experiments as compared with those of Furth and Boon. The leukemogenic effect of carcinogens varies widely for different strains.[4,8] Among those susceptible, synergistic factors such as X-irradiation seem to be of variable significance. The problem of leukemogenesis is further complicated by results such as those obtained with strain C3H mice which were susceptible to the induction of leukemia with estrogens but not with methylcholanthrene.[8,9]

[‡] Subline 12.

[4] Morton, J. J., and Mider, G. B., *Science*, 1938, **87**, 327.

[5] Engelbreth-Holm, J., and Lefevre, H., *Cancer Research*, 1941, **1**, 102.

[6] Law, L. W., and Lewisohn, M., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 143.

[7] Kirschbaum, A., and Strong, L. C., *Am. J. Cancer*, 1939, **37**, 400.

[8] Kirschbaum, A., Strong, L. C., and Gardner, W. U., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 287.

[9] Kirschbaum, A., and Strong, L. C., *Cancer Research*, 1942, **2**, 841.

Summary. Irradiation, when used in conjunction with methylcholanthrene, failed to increase the incidence or to accelerate the onset of carcinogen-induced leukemia in strain Dba and strain F mice.

14550

Simplified Procedures for Ascertaining Concentration of and Bacterial Susceptibility to Penicillin.

A. R. THOMAS, JR., MAX LEVINE, AND GUY R. VITAGLIANO.

From the Medical Corps and the Sanitary Corps, United States Army.

The simplified procedures for determining the concentration of and bacterial resistance to penicillin presented herein, utilize only such equipment and supplies as are already available in all bacteriological laboratories and the results obtained are well within the limits of accuracy reported for the "serial dilution" and "cup assay" technics.

The method is based on the following principles and observations:

1. If a stipulated volume, or concentration, of penicillin solution is placed on the surface of an agar plate previously seeded with a susceptible organism, a circular growth-inhibition zone (area) is produced which is *directly* proportional to the concentration, or volume, respectively, of penicillin applied.

2. If a definite volume (0.01 or 0.02 cc) of a stipulated concentration of penicillin is employed, the growth-inhibition zone (area) will be inversely proportional to the resistance of the test organism.

3. A 4 mm (22-23 gauge) platinum loop was found to deliver a sufficiently constant quantity, from a solution containing 1 to 10 Oxford units of penicillin per cc, to warrant its employment for determination of bacterial resistance to and concentration of penicillin within the limits of accuracy required for routine clinical purposes. The inhibition zones produced by application of such a loop were generally perfectly circular, if care were taken to keep the petri dish flat during incubation.

4. Penicillin is sufficiently stable, in standard culture media stored in a refrigerator, to permit the use of such media for quickly ascertaining the relative susceptibility of bacteria (such as are encountered in lesions and exudates) to this bacteriostatic agent.

The technic is briefly as follows: To 15 cc melted nutrient agar cooled to about 45°C (2-3% blood is added when the test organism is a hemolytic streptococcus) is added 0.05 cc of a 20-24 hr 37°C broth culture of the test organism. The contents are then poured into a Petri dish, allowed to harden and placed in the refrigerator until ready for use (1-4 hours).

To determine the resistance of an organism, 4 mm loops of one or more concentrations of penicillin, in M/50 phosphate buffer at pH 7.2, are placed on the surface of the seeded agar plate (within a few minutes after it is removed from the refrigerator) which is then immediately incubated at 37°C, care being taken to see that the plates are horizontal in order to insure that the inhibition zones will be circular. The diameters of the zones of growth-inhibition are measured the following day (18-22 hr incubation) to the nearest 0.5 mm.

The authors naturally entertained some misgivings as to the constancy and duplicability of the inhibition zones which would be obtained with this loop technic. Repeated observations have demonstrated, however that the results are readily duplicable—the diameters of the inhibition zones produced by a series of loops from a given concentration of penicillin rarely differed by more than 1 mm while generally the error was not greater than ±0.5 mm—and assays performed simultaneously by different observers disclosed coefficients of variability of 2.5 to 4% in the diameters of the growth-inhibition zones which correspond to a variability of 10-16% in the assay of penicillin potency. Such errors are well within the limits of 15-25% claimed for the "serial dilution" and "cup assay" procedures. It is suggested that development of capillary pipettes which will deliver 0.015 or 0.02 cc would further enhance the accuracy of the technic presented.

TABLE I.
Relation of Concentration of Penicillin to Zone of Growth-Inhibition.

Conc. of penicillin (Oxford units per ml)	Zone of inhibition on seeded agar* (Diameter in mm)
10.2	29.8 ± 0.3
5.1	25.7 ± 0.3
2.55	20.2 ± 0.6
1.28	16.8 ± 0.3
0.64	12.7 ± 0.6
0.32	8.0 ± 0.4

* Produced by 4 mm loop employing the Oxford strain *Staphylococcus aureus* as inoculum.

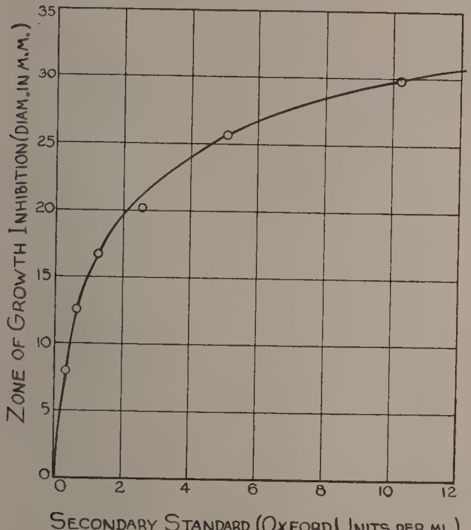

Fig. 1.
Effect of concentration of penicillin (4 mm loop) on zone of inhibition of Oxford strain of Staphylooccus.

In Table I are shown the diameters of the inhibition zones obtained when 4 mm loops of various concentrations of a standard penicillin (kindly furnished by Dr. J. W. Foster[1] of Merck & Co.) were placed on nutrient agar which had been seeded with the Oxford strain *Staph. aureus.* The accompanying photograph (Plate 1) illustrates the appearance of inhibition zones produced against *Staph. aureus* by a 4 mm loop of various concentrations of penicillin.

It will be noted from Fig. 1, where the inhibition zones are plotted against the concentrations of penicillin applied, that the points fall along a smooth curve which will be employed as a curve of reference. Penicillin, as employed therapeutically, is generally prepared so as to contain 5000 Oxford units per cc. If this solution is further diluted 500 (or 1000) fold in M/50 phosphate buffer at pH 7.2, a 4 mm loop of the resulting solution (presumably containing 10 (or 5) units per cc) placed on the surface of agar seeded with the Oxford strain of *Staph. aureus* and the plate incubated at 37°C for 20 hours, the inhibition zone produced, when projected on to the curve in Fig. 1, will show the concentration of the penicillin being employed.

This reference curve is particularly useful for studies on the stability, or rate of destruction of penicillin. With an initial concentration of approximately 10 (or 100) Oxford units of penicillin per cc, the rate of decrease in potency may be easily followed by (1) placing a 4 mm loop of the test solution (diluted 1 to 10 if the initial concentration was 100 units) at desired intervals, on to seeded agar, (2) measuring the diameter of the growth-inhibition zones after 18-22 hours incubation at 37°C and (3) reading off from the reference curve the corresponding penicillin concentrations. A single seeded agar plate may be employed for 4 to 6 determinations and preparation of an extensive series of dilutions of the material under observation (such as would be required for either the

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[1] Foster, J. W., and Woodruff, H. Boyd, *J. Bact.*, 1943, **46**, 187.

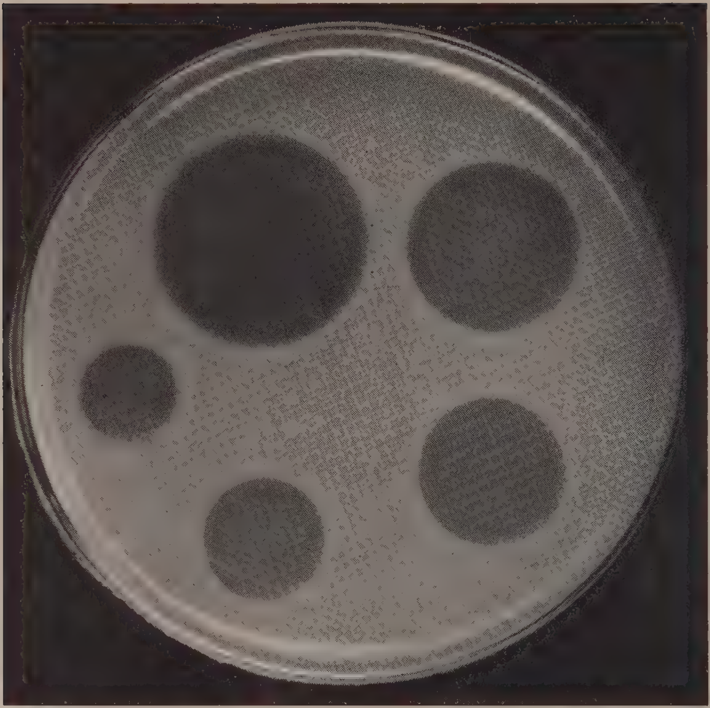

PLATE 1.
Inhibition zones produced by 4 mm loops of penicillin solutions containing 10, 5, 2.5, 1.25, and 0.63 Oxford units per cc, placed on the surface of agar seeded with the Oxford strain of *Staph. aureus.*

TABLE II.
Inhibition Zones Produced by 4 mm Loop of 10 Units of Penicillin on Agar Seeded with Staphylococci of Various Degrees of Resistance to Penicillin in Broth.

	Conc. of penicillin (Oxford units per ml)		
No. of Obs.	Permitting growth	Preventing growth	Zone of inhibition on seeded agar (Diam. in mm)
16	0.02	0.04	28.9 ± 1.2
9	0.04	0.08	24.6 ± 1.3
7	0.08	0.16	18.9 ± 1.0
4	0.16	0.32	17.1 ± 0.3
5	0.32	0.64	14.0 ± 0.8

serial dilution or cup assay technics) is eliminated.

In Table II are shown the inhibition zones produced when 4 mm loops of M/50 phosphate buffer (pH 7.2) containing 10 units of penicillin per cc, were placed on agar seeded with staphylococci of various degrees of resistance to penicillin. By plotting the diameters of the inhibition zones against the concentrations of penicillin permitting (or preventing) growth in broth, a curve is obtained which may be utilized to convert growth-inhibition zones produced on agar seeded with a test organism into the probable concentrations of penicillin which would permit (or prevent) growth of the organism in broth. In Table III, which is derived from such a curve, are shown the probable inhibiting concentrations of penicillin in broth corresponding to growth-inhibition zones (at intervals of 2 mm) obtained on seeded agar.

In practice, a knowledge of the exact resistance of an organism to penicillin is not as significant as is the information whether a suspected organism is so susceptible (or resistant) that the concentration attainable in the blood stream may reasonably be expected

TABLE III.
Relation of Inhibition Zone Produced* on Agar Seeded with Staphylococci to Probable Concentration of Penicillin Which Will Permit (or Prevent) Growth in Broth.

Inhibition zone on seeded agar (Diameter in mm)	Probable conc. (Oxford units per cc) in beef extr. broth	
	Permitting growth	Preventing growth
29	0.02	0.04
27	0.03	0.06
25	0.04	0.08
23	0.05	0.10
21	0.07	0.14
19	0.09	0.18
17	0.14	0.28
15	0.21	0.42

* When a 4 mm loop of 10 units of a penicillin solution in M/50 phosphate buffer (pH 7.2) is placed on the surface of chilled seeded agar.

to be effective (or ineffective) against the organism in question. If growth in 0.2 units were taken as the limit of resistance of staphylococci or other organisms amenable to penicillin treatment, then, if inhibition zones less than 15 mm in diameter were obtained by the procedure described above, the organisms in question would be considered too resistant to warrant satisfactory results when dealing with systemic infections.

It has been observed that penicillin, when placed in bacterial culture media, such as thioglycollate broth or blood agar, deteriorates quite rapidly at 37°C, but when stored at ice-box temperature the decrease in potency was insignificant, even after a period of several weeks. This would indicate that it should be feasible to employ blood agar or other suitable media containing penicillin, if stored in a refrigerator, to ascertain, *simultaneously*, the nature of the organisms present in a lesion or exudate and, within desired limits, the probable resistance of these organisms to penicillin. Observations with several strains of *Staph. aureus* disclosed that inhibiting concentrations of penicillin in broth were practically the same as those obtained when penicillin was incorporated into blood agar media.

It has been the practice in the laboratory of the Brooke General Hospital for several months to suspend exudates in small quantities (0.2 to 0.3 cc) of physiological salt solution (or M/50 phosphate buffer at pH 7.2) and streak out a loop of this suspension on blood agar and blood agar containing 0.04, 0.1, and 0.25 units of penicillin per cc of media. The types of organisms growing on the media containing various concentrations of penicillin were then allocated tentatively, with respect to penicillin susceptibility, as shown in Table IV. When the various types of colonies developing on the blood agar without penicillin were fished and examined for penicillin resistance by the dilution and inhibition-zone technics, the results correlated well with the tentative allocations of the organisms as respects their susceptibility to penicillin and with the clinical observations.

Summary. Technics requiring only such equipment and supplies as are already available in any bacteriological laboratory are presented (1) for developing reference curves and tables which may be used (a) for determination of penicillin concentration of solutions employed therapeutically and (b) for following readily the rate of decrease of penicillin potency, (2) for converting growth inhibition zones (for staphylococci) on seeded agar to probable inhibiting concentrations of penicillin in broth, and (3) which make feasible the tentative allocation of bacteria, as respects their susceptibility to penicillin, simultaneously with the determination of the types of organisms encountered, in lesions and exudates, within 15-18 hours after collection of specimens.

TABLE IV.
Relation of Growth of Organisms from Exudates on Blood Agar Containing Penicillin to Anticipated Therapeutic Response.

Susceptibility to penicillin	Conc. penicillin in blood agar (Oxford units per cc)				Anticipated response to therapy*
	None	0.04	0.1	0.25	
	Growth on blood agar (24 hrs 37°C)				
Very high	+	—	—	—	Satisfactory
High	+	+	—	—	"
Moderate	+	+	+	—	Questionable
Resistant	+	+	+	+	Unsatisfactory

* As respects amenability to systemic infection.

14551

Effects of Testosterone on Genital Organs of Female Rats Treated from Date of Birth.*

G. L. LAQUEUR.

From the Department of Obstetrics and Gynecology, Stanford University School of Medicine, San Francisco, California.

The effect of testosterone propionate (T.p.) upon the ovaries of female rats has been previously reported from this laboratory. It was shown that T.p. prevented the formation of corpora lutea in adult as well as in 21- to 23-day-old immature rats and that follicular development was not arrested.[1] It was also reported that T.p. enhances and maintains the function of corpora lutea of the rat provided early corpus luteum formation is present at the time of the first injection.[2] These observations are in contrast to those of other workers who report the formation of corpora lutea in immature rats[3,4,5] and the findings of cystic corpus luteum tumors in rats treated with T.p. from birth.[6] The present report summarizes our findings in female rats treated with T.p. from the day of birth for periods ranging from 7 to 232 days.

Of a total of 61 female rats of the Wistar strain, 46 survived the treatment. The animals, beginning with the day of birth, were given subcutaneous injections of 1 mg (0.1 cc) of testosterone propionate dissolved in oil 3 times weekly. The pertinent data are given in Table I. At autopsy, the ovaries, tubes, and periovarian tissue were removed *in toto*, and, after fixation and embedding, sectioned serially until the ovaries were demonstrable. Hematoxylin-eosin was used routinely, supplemented by Weigert-van Gieson, Gram, and Sudan III stains. Representative sections of uteri, vaginæ, and other organs were examined.

The ovaries, in serial sections, were found to be atrophic, and follicular development was arrested just beyond the stage of antrum formation although in one animal a few large follicles were found. Corpora lutea were uniformly absent (Fig. 1). There was atrophy of the stromal and thecal cells, some of the

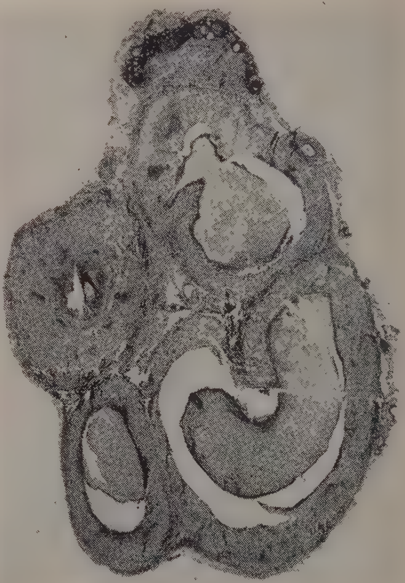

FIG. 1.
Rat 51721, 40 mg in 92 days. Unilateral right tubal abscess. Note atrophic ovary at upper pole.

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* Supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine. Testosterone propionate (Perandren) was generously supplied by the Ciba Pharmaceutical Products, Inc., Summit, N.J. My thanks are due to Mrs. Agnes Chinn, Mr. V. Montesclaros, and Mr. P. Lassegues for their technical help.

[1] Laqueur, G. L., and Fluhmann, C. F., *Endocrinology*, 1942, **30**, 93.

[2] Fluhmann, C. F., and Laqueur, G. L., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 223.

[3] Hohlweg, W., *Klin. Wchnschr.*, 1937, **16**, 586.

[4] Salmon, U. J., *Endocrinology*, 1938, **23**, 779.

[5] Nathanson, I. T., Franseen, C. C., and Sweeney, A. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 385.

[6] Shay, H., Gershon-Cohen, J., Paschkis, K. E., and Fels, S. S., *Endocrinology*, 1939, **25**, 933.

TABLE I.
Incidence of Tubal Abscesses in Female Rats Treated from Birth with Testosterone.

No. of rats	Total dosage, mg	Period of inject., days	Avg wt, g	Tubal abscesses Present	Absent
3	4	7	12.4	0	3
3	8	17	24.7	0	3
3	12	27	58.0	0	3
4	16	36	81.7	2	2
4	20	45	119.7	1	3
3	25	57	143.0	1	2
4	30	68	181.5	1	3
3	40	92	223.7	2	1
3	50	116	186.7	1	2
2	60	143	190.0	1	1
3	70	165	228.0	2	1
2	80	184	244.5	2	0
2	90	207	246.0	1	1
7	100	232	218.0	4	3

latter showing the nuclear derangement described as deficiency cells.[7] With the larger dosages, a significant number of follicles were altered so that they resembled testicular tubules as described in detail by Marx.[8]

Tissue masses of various sizes were found in the region of the adnexa in 18 animals, 12 bilaterally and 6 unilaterally. These lesions grossly resembled those described by Shay *et al.*[6] Although there was no direct proportionality between the total dosage and the presence of these lesions, none were found in the animals receiving less than 16 mg. Histologically, the masses represented various stages of salpingitis, mesosalpingitis, and periovarian cellulitis. In all acute and subacute lesions, Gram stain demonstrated a variety of bacteria. In the older lesions granulation tissue had walled off the abscesses, and innumerable large mononuclear cells, occasionally forming multinucleated giant cells, were seen. Frozen sections stained with Sudan III demonstrated abundant fatty material in the mononuclear cells. Acute as well as chronic stages of salpingitis were seen in the same section, and in more than half of them parts of normal fallopian tubes were found adjacent to the abscess walls.

The ovaries of rats with tubal abscesses were not grossly identified, but histologically they showed the changes already described. In a few instances, however, fibrosis was marked suggesting a previous oophoritis. In 3 rats, this was in the acute stage, the periovarian space being filled with an inflammatory exudate.

In rats with pyosalpinges, the uteri and vaginæ of 6 were distended by secretion, leucocytes, and a few bacteria while in 12 rats there was no uterine involvement. On the other hand, pyometra were found in 3 rats without an accompanying salpingitis. In all animals treated longer than 36 days, the vagina was distended by strongly basophilic mucoid material and leucocytes. This mucoid material was presumably produced by secretory cells which were frequently found among the squamous epithelial cells. Although the vaginal orifice was not regularly inspected during the course of the experiment, it was found at autopsy that in 42 of the 46 rats the vagina was closed.

Examination of the tubes with no abscess formation showed uniform secretory changes in the epithelium accompanied by a mild leucocytic invasion, but no bacteria were demonstrated. Hence, the formation of abscesses was probably secondary, and superimposed upon preceding secretory activity of the tubal epithelium. The source of the bacterial invasion was not conclusively determined. No other inflammatory lesions, even at the site of injection, were found in these animals.

The ovarian changes reported here are in accord with those reported by Mazer and Mazer[9] and by us[1] where treatment was begun in slightly older but still immature rats. The development of tubal lesions in rats injected from birth is of interest in connection with the observations of Green and Ivy[10] and Hamilton and Gardner[11] who found pyometra and distention of the vagina in rats subjected to an androgenic stimulus during the last third of intrauterine growth.

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[7] Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinology*, 1933, **17**, 494.

[8] Marx, L., *J. Exp. Zool.*, 1942, **91**, 365.

Conclusions. 1. Testosterone propionate injected into female rats from the day of birth arrests follicular development beyond the stage of the antrum and prevents corpus luteum formation. 2. These findings extend our previous observations on adult and 21-day-old rats, and it is therefore concluded that testosterone in the doses used prevents luteinization of the rat's ovary at any age. 3. When treatment is initiated on the day of birth, there is an arrest of follicular development and a partial transformation of the follicles into seminiferous tubule-like structures, not observed in our previous study with older rats. 4. Inflammatory lesions representing various stages of salpingitis, mesosalpingitis, and periovarian cellulitis appeared in about 40 per cent of the experimental animals. 5. The formation of tubal abscesses, caused by secondary bacterial invasion, is preceded by the stimulation of secretory changes in the tubal epithelium.

Since the paper was submitted for publication, a preliminary report by H. B. Hale and C. K. Weichert appeared in the March issue of these PROCEEDINGS (1944, **55**, 201), in which the authors report on ovarian tumors in adult rats following prepuberal administration of estrogens. Grossly, these tumors resembled those reported by Shay *et al.*[6]

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[9] Mazer, M., and Mazer, Ch., *Endocrinology*, 1939, **24**, 175.

[10] Greene, R. R., and Ivy, A. C., *Science*, 1937, **86**, 200.

[11] Hamilton, J. B., and Gardner, W. U., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 570.

14552

A Method of Blood Replacement in the Rat.

DONALD H. LAUGHLAND. (Introduced by Hardolph Wasteneys.)

From the Department of Biochemistry, University of Toronto, Toronto, Canada.

In the course of experiments in this laboratory it became necessary to remove a large proportion of the blood of a rat and to replace it rapidly with other blood. Preliminary experiments showed that the readily accessible vessels in the rat, such as the jugular, femoral, and tail veins, are not suitable for the rapid withdrawal and replacement of blood. It was found, however, that the inferior vena cava is a very satisfactory site for the performance of this operation, and that rats recover rapidly in spite of the surgical procedure which is necessitated by the use of this vessel.

Method. The rat (wt. 150-250 g) is anesthetized, the abdomen is incised, and the inferior vena cava is exposed. A 20-gauge hypodermic needle which has been bent at an angle of 45° about 0.5 cm from the tip is inserted into the vessel and is held firmly in position by means of a clamp. The rat is heparinized (25 units per 100 g body wt) by attaching to the needle a syringe containing heparin solution (Connaught Laboratories, Toronto; 1000 units of heparin per ml), drawing 1 ml of blood into the syringe and then reinjecting it. An amount of blood not exceeding 5 ml is then drawn into the syringe. The syringe is replaced by another containing the same volume of heparinized blood and this is then injected. After the replacement process has been carried out the required number of times, the vessel around the site of entry of the needle is grasped with smooth blunt forceps and the needle is carefully withdrawn. A few drops of a solution of thrombin*

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* The writer wishes to thank Parke, Davis and Company for a gift of a supply of thrombin.

in saline are then placed on the bleeding point and the pressure of the forceps is gradually released. Under these conditions bleeding from the vessel can readily be prevented. The incision in the abdominal wall is then closed with plain No. 00 catgut. Surgical silk is used to close the skin incision and these sutures are removed 7 days later.

Application of the method. The procedure described above was first studied with 6 rats (wt range 158-250 g, average wt 199 g). In each experiment 5 ml of blood were withdrawn and reinjected several times at short intervals. After showing small losses in weight during the 48 hours following the operation the rats gained steadily in weight.

A second series of experiments with 6 rats was performed. In each experiment 4 withdrawals of 3 ml of blood were made over a period of 4 minutes. After each withdrawal 3 ml of pooled heparinized blood from normal rats of the same strain were injected. The average red cell counts of these animals on the 7th and 14th days after the operation did not differ significantly from those found on the 6th and 2nd days before the operation. The rats recovered rapidly and the internal organs appeared normal on gross examination when the animals were killed 14 days after the operation. The average weight of the rats was 160 g at the time of operation and 14 days later it was 200 g. A control series of 6 rats increased in average weight from 161 g to 195 g during the same period.

The average blood volume of the rat has been reported as 4.3 ml,[1] 6.13 ml,[2], and 7.4 ml[3] per 100 g of body weight. In the present work experiments using the Evans blue technic gave values of 6.7 ml and 6.5 ml of blood per 100 g of body weight for a male and a female rat weighing 150 g and 170 g respectively. After four 3-ml portions of blood from each of these animals had been withdrawn and had been replaced by blood from other rats by means of the procedure described above, the concentration of dye in the plasma indicated that 72% of the original blood had been replaced in both cases. The calculated replacements based on the determined blood volumes are 76% and 72% respectively.

In addition to experiments of the type which have been described it has been found possible to use the method in the performance of cross-transfusion experiments. In such experiments the same amount of blood is drawn from two animals simultaneously, the syringes are interchanged and the blood is injected.

Summary. A method of rapid blood replacement in the rat is described in which the inferior vena cava is used as the channel of withdrawal and replacement of the blood. The results of experiments in which this method was applied are described.

The author wishes to thank Dr. Leslie Young for the advice which he gave during the course of the experiments.

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[1] Griffith, J. Q., and Campbell, R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 38.

[2] Scott, J. M. D., and Barcroft, J., *Biochem. J.*, 1924, **18**, 1.

[3] Went, S., and Drinker, C. K., *Am. J. Physiol.*, 1929, **88**, 468.

14553 P

A Standard Method of Producing Shock in Dogs by a Single Massive Hemorrhage.*

William W. Walcott. (Introduced by M. I. Gregersen.)
From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

The problem of producing consistently fatal shock in dogs by a single massive bleeding is complicated by the great variability in the tolerance of these animals to hemorrhage. If bled a fixed volume per unit of body weight, the results are inconstant due to the wide range in the blood volume/body weight ratio in this species. Removal of a fixed percentage of the measured blood volume is likewise unreliable because of the variability in the magnitude of compensatory replacement of both plasma and cells (*i.e.*, with tissue fluid and splenic reserve). Dogs subjected to a single massive hemorrhage by either method may compensate sufficiently to restore the blood volume above the critical level where progressive shock develops or they may fail to compensate and die immediately. The problem is to bleed in such a way that the outcome will be fatal after a period of time sufficiently long to produce the characteristic changes associated with hemorrhagic shock.

The method which has been developed to meet this problem is based on the assumption that the tolerance of any given animal to hemorrhage may be gauged by the bleeding volume. The unanesthetized dog is subjected to a rapid blood loss practically equivalent to its bleeding volume by allowing the blood to run freely from a femoral arterial cannula (as large as compatible) until it flows only in driblets and the animal is in a state of functional decerebrate rigidity. The first 25% of this volume having beeen collected in large syringes containing heparin is immediately returned through the femoral vein or via the bleeding tube and arterial cannula. In the present series 12 ml blood samples were taken for analysis every half-hour after the hemorrhage. The animals were kept in the supine position throughout the experiment.

The average time for the determination of the bleeding volume was 7 minutes, and for the reinfusion, 1¾ minutes. The average survival time for 21 dogs in which shock was induced by this method was 3 hours, 40 minutes (range 1¼ to 5¾ hours, standard deviation 18 minutes). The bleeding volumes of these animals averaged 62.4 ml per kilo of body weight, or 70.7% of the measured blood volume. Blood volume determinations on 10 dogs made 1 to 3 hours after hemorrhage showed an average reduction of 45.3% from the control value (range 40.4 to 47.5%). This is in agreement with the results from a larger series of dogs bled by removing a fixed quantity of blood per unit of body weight. From the unpublished data[1] of these experiments it was established that the animals which died as the result of a single massive hemorrhage had suffered a blood volume reduction of 40% or greater.

Unanesthetized dogs which were subjected to the loss of 75% of their bleeding volume by the method described here showed the clinical course, the pathology, and all the changes in blood chemistry characteristic of hemorrhagic shock.

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* This work was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and Columbia University.

[1] Walcott, William W., to be published.

14554 P

Effect of Veratrone on Urine Volume and Urea Clearance in Hypertensive Toxemias of Pregnancy.

J. Robert Willson. (Introduced by William J. Dieckmann.)

From the Department of Obstetrics and Gynecology, The University of Chicago, and the Chicago Lying-In Hospital.

Although the use of veratrum viride, a combination of alkaloids from the rhizomes of the green hellebore, has for the most part been discontinued, the recent report of Bryant and Fleming[1] suggests that it may have some value as an adjunct to the treatment of eclampsia.

The predominant physiological effect of the drug in animals is central vagus stimulation resulting in a marked fall in pulse rate and blood pressure. Recently an effect on the peripheral vascular system was demonstrated by Willson and Smith.[2] They were able to produce a fall in blood pressure without change in pulse rate when the drug was given to vagotomized dogs. Likewise, an increased perfusion rate through the kidney, the isolated hind leg, and ear of rabbits occurred when the drug was added to the perfusion fluid.

Because information concerning the effects of the drug in the human being is so limited it seems necessary that a complete investigation of the action of veratrum viride on the blood vascular system in the pregnant woman be made. This report concerns the preliminary results of the first of a number of such studies being carried out by us.

Method. The subjects were all prenatal patients confined to the hospital and at complete bed rest. On the morning of the test 200 cc of water were given by mouth every half hour starting at 7:00 A.M. until a total of 1800-2000 cc had been taken and urine specimens were taken by catheter at half hour intervals. The bladder was emptied completely each time. A control urea clearance was started at the end of one hour and the drug, Veratrone, a preparation of veratrum viride for parenteral use, was administered shortly after the completion of the initial test period. Usually at least 2 more clearances were run after the drug had been given; however, in some instances this was impossible because of suppression of urinary output. Only one blood specimen was taken because no appreciable change in the blood constituents was observed. Blood pressures were all taken by one individual with a mercury manometer.

Results. Twelve tests have been run on 10 subjects: 5 pre-eclamptics, 4 hypertensives, and one normal. The study was repeated on the seventh postpartum day on 2 patients.

1. *Effect on Blood Pressure.* A drop in both systolic and diastolic blood pressures followed the administration of veratrone in each instance. The effect was first noted in about 10 minutes and the most pronounced effect on blood pressure occurred about 2-2½ hours after the drug was given. A more marked reaction occurred with larger initial dosages and an increasing effect occurred with subsequent injections.

2. *Effect on Urine Volume.* Associated with the drop in blood pressure was noted a decrease in urine output. This was more marked in the pre-eclamptics than in the hypertensives. One of the former was anuric for 2 hours and another for one hour after a depression of systolic blood pressure from 170 mm Hg. to 100 mm Hg.

3. *Effect on Urea Clearance.* In most instances the urea clearance was decreased when measured during the periods of low blood pressure. The decrease was progressive in the periods studied and in one instance the depression amounted to over four-fifths of the control period.

These changes are illustrated in Fig. 1.

Discussion. The administration of veratrone to patients with the hypertensive tox-

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[1] Bryant, R. D., and Fleming, J. G., *J. A. M. A.*, 1940, **115**, 1333.

[2] Willson, J. R., and Smith, R. G., *J. Pharm. and Exp. Therap.*, 1943, **79**, 208.

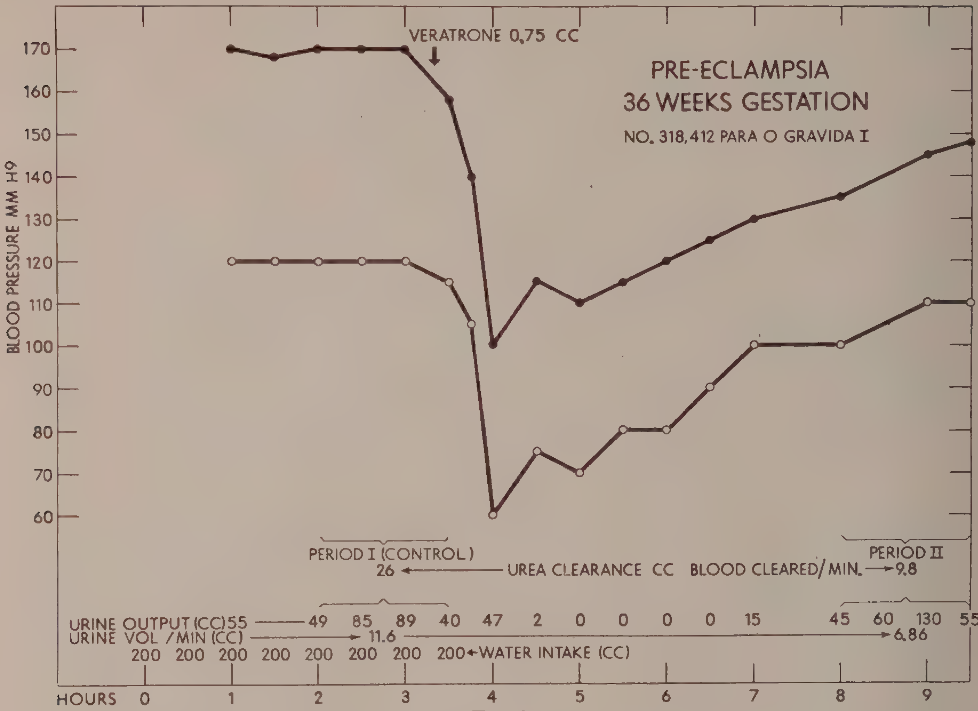

Fig. 1.

emias of pregnancy not only lowers the blood pressure but also decreases kidney function as demonstrated by a depression both in urine volume and urea clearance. It is the opinion of the author that this is the result of two factors: the fall in blood pressure and an alteration in renal dynamics resulting from the action of the drug on the vascular system. Studies on kidney blood flow and glomerular filtration will aid in determining the answer to this problem.

14555

Effect of Xyloquinone in Normal Dogs and Dogs with "Neurogenic Hypertension."

Paul W. Schafer. (Introduced by Lester R. Dragstedt.)
From the Department of Surgery, University of Chicago.

Some evidence supports the view that hypertension observed in experimental renal ischemia is due to faulty deamination of amino acids.[1,2] By the action of decarboxylases pressor amines are formed in the kidney as intermediary products in the metabolism of certain amino acids. In the kidney with a normal blood supply these amines are deaminized to inert substances through the action of the enzyme, amine oxidase. However, in renal ischemia this latter enzyme is not active and

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[1] Holtz, P., and Luedtke, K., *Arch. Exp. Path. u. Pharm.*, 1939, **191**, 87.

[2] Bing, R. J., *Am. J. Physiol.*, 1941, **132**, 497.

the pressor amines are not destroyed and may possibly be the basis for the hypertension observed in such conditions. Soloway[3] has shown that certain pressor amines can be inactivated both *in vivo* and *in vitro* by the action of certain quinone precursors, presumably by the formation of amine-inactivating quinones. Following this work Friedman and Soloway[4] demonstrated the marked antipressor effect of 3 paraquinones and one orthoquinone in rats made hypertensive by perinephritic scars. These quinones were effective in lowering blood pressure when given both orally and subcutaneously and were found to produce no change in the blood pressure of normal rats. The exact mode of action of quinones in lowering blood pressure is not known but the most probable theory is that they act through an effect on circulating amines.

Aside from the elevated blood pressure common to both, few points of similarity exist between Goldblatt[5] and "neurogenic"[6] types of hypertension. "Neurogenic" hypertension has been produced in dogs in this laboratory for the past 5 years by Grimson[7] and the author.[8] The procedure has been previously reported and consists essentially of section of carotid sinus and aortic depressor proprioceptor nerves. We have held the view that the elevated blood pressure observed following proprioceptor depressor neurotomy was due primarily to marked increased peripheral vasoconstriction caused by uninhibited action of vasopressor impulses.

Since Friedman and Soloway have demonstrated marked blood pressure reducing properties of certain quinones in rats made hypertensive by renal ischemia we were interested in testing the effect of the most potent of these chemicals on our dogs made hypertensive by a fundamentally different method.

Procedure. Seven adult male dogs were used, 4 with chronic "neurogenic" hypertension and 3 normal controls. The 4 hypertensive dogs had had a sustained elevation of blood pressure for 2, 19, 30, and 32 months respectively with mean arterial blood pressure averages and deviations as follows:

Dog No. 49—Avg, 230 mm Hg; range, 210 to 260 mm Hg.
Dog No. 735—Avg, 235 mm Hg; range, 226 to 240 mm Hg.
Dog No. 139—Avg, 257 mm Hg; range, 220 to 288 mm Hg.
Dog No. 804—Avg, 208 mm Hg; range, 186 to 226 mm Hg.

All 7 dogs were given a subcutaneous injection of 10 cc of a 0.1% solution of 2,5-dimethyl paraquinone (xyloquinone) in sterile normal saline solution per kg of body weight. Mean femoral arterial blood pressure determinations were made at intervals as indicated in Table I. After the 4 hypertensive animals had recovered from the effect of the xyloquinone injection they were each given a subcutaneous injection of 10 cc of a sterile normal saline solution per kg of body weight and blood pressure determinations made at intervals as indicated in Table I. The average blood pressure changes for each group are plotted in Fig. 1.

Results. No significant changes were observed in the mean arterial blood pressure of normal dogs injected with xyloquinone or in hypertensive dogs injected with normal saline solution in the amount indicated above. Hypertensive dogs given a single subcutaneous injection of 10 mg of xyloquinone per kg of body weight were observed to have a precipitous reduction of blood pressure by 120 mm Hg., maximal at 6 hrs, and requiring 4 days for complete recovery. No changes could be detected in the behavior of any of these animals and the local sites of injection did not show signs of inflammation. Body temperature and weight remained normal. Smaller amounts of xyloquinone than that indicated above have been used with less marked and less prolonged effect. Larger amounts, given deliberately to produce signs of overdosage,

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[3] Soloway, S., and Oster, K. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 108.

[4] Friedman, B., and Soloway, S., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 195.

[5] Goldblatt, H., Lynch, J., *et al.*, *Am. J. Path.*, 1933, **9**, 1942.

[6] Heymans, C., and Bouchaert, J. L., *C. R. Soc. de biol.*, 1931, **106**, 471.

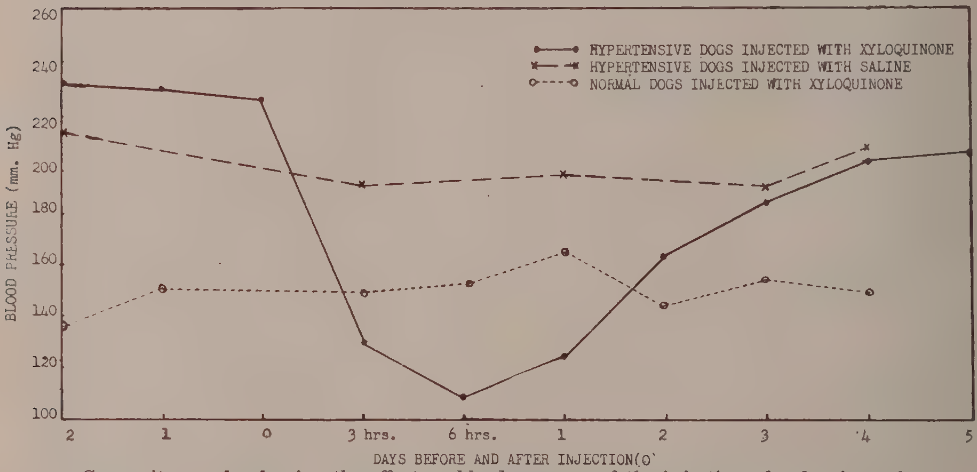
[7] Grimson, K. S., *Arch. Surg.*, 1941, **43**, 284.

[8] Schafer, P. W., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 327.

TABLE I.
Blood Pressure Readings Before and After Injection of Xyloquinone in Normal Saline Solution into Four Hypertensive Dogs (Group I), and into Three Normal Dogs (Group III). Blood Pressure Readings Before and After the Injection of an Equal Volume of Normal Saline into Three Hypertensive Dogs (Group II) Are Also Shown.

	Blood pressure (mm Hg) on days before and after injection										
Dog No.	2	1	0	3 hrs	6 hrs	1	2	3	4	5	6
Group I—Hypertensive Dogs Injected with Xyloquinone (10 cc .1% sol./kg body weight).											
49		240	220	120	100	144	170	190		240	230
735		226		106	90	80	176	206		170	200
139		210	220	128	130	156	174	198		180	200
804		226	200	170	122	126	140	146		216	186
Group II—Hypertensive Dogs Injected with Normal Saline (10 cc/kg body weight)											
49	260			230		240		230	250		
735	188			160		140		188	180		
139	192			186		210		152	190		
Group III—Normal Dogs Injected with Xyloquinone (10 cc .1% sol./kg body weight).											
296	140	165		160	170	200	160	160	166		
297	130	140		140	150	140	132	136	126		
298	140	150		150	150	160	146	170	160		

FIG. 1 - EFFECT OF XYLOQUINONE ON THE BLOOD PRESSURE OF HYPERTENSIVE AND NORMAL DOGS.



Composite graphs showing the effect on blood pressure of the injection of xyloquinone into hypertensive dogs (Group I) indicated by the solid line, and into normal dogs (Group III) indicated by the dotted line. The effect of injection of saline solution into hypertensive dogs Group II) is indicated by the dashed line.

have been found to cause marked intravascular hemolysis, hemoglobin cast occlusion of renal tubules, uremia and death.

Discussion. Since xyloquinone is effective in lowering the blood pressure of rats made hypertensive by renal ischemia and dogs made hypertensive by proprioceptor depressor neurotomy one has to ascribe to it either a non-specific depressor action or else consider the possibility that both types of hypertension may have similar humoral aspects. The fact that the blood pressure of normal rats and normal dogs is not changed by this chemical and that the animals present no untoward signs or symptoms make it seem unlikely that the effect is non-specific. Of obvious interest would be the effect of this agent on the blood pressure of patients with "essential hypertension" but the hazard of hemolytic reactions should cause this problem to be approached with due care.

Summary. 1. 2,5-dimethyl paraquinone was found to cause a marked reduction in the blood pressure of dogs made hypertensive by proprioceptor depressor neurotomy. 2. No significant changes were observed in the blood pressure of normal dogs given an amount of this chemical sufficient to cause a marked effect in hypertensive dogs.

The author wishes to acknowledge the technical assistance of Carl Laestar.

14556

Ineffectiveness of Sodium Succinate in Control of Duration of Barbiturate Anesthesia

HENRY A. LARDY, R. GAURTH HANSEN, AND PAUL H. PHILLIPS.
From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Recently Soskin and Taubenhaus[1] have reported the effectiveness of sodium succinate in shortening the duration of barbiturate anesthesia in rats. Succinate was also reported to be of some benefit as an antidote for lethal doses of pentobarbital. The impetus for their experimental work was the observation of Quastel and Wheatley[2] that barbiturates depress the respiration of brain tissue *in vitro* when glucose, pyruvate, or lactate are the substrates, but that succinate oxidation is not inhibited. We had found[3] that in tissue preparations which rapidly oxidized succinate *in vitro* the oxidation was not coupled with phosphorylation. Coupling of oxidation with phosphorylation is, as far as is known, the only mechanism by which the organism may derive energy from the oxidation of metabolites. While we have no reason to doubt that succinate oxidation *in vivo* is coupled with phosphorylation the results with tissue preparations suggested that the alterations in tissue during the preparation for manometric experiments are such that the rate of oxidation of substrates *in vitro* does not necessarily indicate the relative importance of that substrate as an energy source *in vivo*.

The observations of Soskin and Taubenhaus seemed of sufficient importance to merit further investigation. Upon repetition of their work we were unable to confirm their conclusion that the administration of sodium succinate shortens the duration of both pentobarbital and amytal anesthesia in rats.

Experimental and Results. Male rats of the Sprague-Dawley strain, weighing from 200 to 250 g and maintained on stock rations, were the experimental subjects. The sodium salts of the barbiturates in aqueous solution were injected intraperitoneally. Where succinate was administered it was injected as an aqueous solution containing 200 mg of sodium succinate per cc. Equivalent amounts of malate were given some animals. Both succinate and malate were injected 1 to 5 minutes after the barbiturate. Duration of anesthesia was measured from the time of disappearance of the eyelid reflex until the animal arose and moved about the cage. Other experimental conditions and the results obtained are shown in Table I.

In Experiment 1 neither succinate nor malate, administered intramuscularly following the intraperitoneal injection of pentobarbital, significantly affected the duration of anesthesia. Similar results were obtained in Experiment 2 with pentobarbital and in Experiment 3 with amytal.* The experimental conditions in these experiments were very similar to those under which Soskin and Taubenhaus obtained the most dramatic

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[1] Soskin, S., and Taubenhaus, M., *J. Pharm.*, 1943, **78**, 49.

[2] Quastel, J. H., and Wheatley, A. H. M., *Proc. Roy. Soc. London*, 1932, B**112**, 60.

[3] Boyer, P. D., Lardy, H. A., and Phillips, P. H., unpublished data.

* We are indebted to Dr. O. S. Orth for supplying the amytal used in these experiments.

TABLE I.
Effect of Succinate and Malate on the Duration of Barbiturate Anesthesia in Rats.

Exp. No.	Barbiturate	Nutritive state	No. of rats	Further treatment	Duration of anesthesia* min.
1	Na Pentobarbital 3.2 mg/100 g wt	Fasted 24 hrs	16 16 15	Control Succinate, 50 mg/100 g I.M.† Malate, 55 ” ” ”	28 ± 11 33 ± 10 26 ± 7.4
2	Na Pentobarbital 3.2 mg/100 g	” 16 ”	14 14	Control Succinate, 50 ” ” ”	18 ± 6.9 18 ± 3.1
3	Na Iso-amyl Ethyl Barbiturate 5 mg/100 g	” 16 ”	13 13	Control Succinate 50 ” ” ”	21 ± 9.9 26 ± 10
4	Na Pentobarbital 3.2 mg/100 g	Well fed	3 4 4	Control Succinate, 100 ” ” I.P.‡ Malate, 111 ” ” ”	32 ± 13 69 ± 6.2 90 ± 4.0
5	Na Pentobarbital 6.5 mg/100 g	” ”	2 3 2	Control Succinate, 100 ” ” ” Malate, 111 ” ” ”	120 165 187

* Mean and standard deviation of the mean.
† I.M.—Intramuscularly.
‡ I.P.—Intraperitoneally.

response with succinate. The animals had been fasted and comparatively large doses of succinate were administered.

In Experiments 4 and 5 succinate and malate were administered intraperitoneally. Although it is recognized that metabolites injected intraperitoneally are apt to be absorbed into the portal circulation and deposited in the liver before they can reach the brain, experimental conditions were arranged to have this deposition at a minimum. The animals were well fed and, in addition, large doses of the metabolites were injected. In no case did succinate shorten the duration of anesthesia. The apparent prolongation of the anesthesia by succinate and malate in experiments 4 and 5 is probably not significant because of the limited number of animals.

Summary. The intramuscular administration of sodium succinate to fasted rats under pentobarbital or amytal anesthesia did not significantly shorten the duration of the anesthesia. Similarly the intraperitoneal administration of succinate or malate to a limited number of fed rats under pentobarbital anesthesia did not significantly influence the duration of anesthesia. We were unable to confirm Soskin and Taubenhaus' observations that succinate is an effective agent in the control of barbiturate anesthesia.

14557 P

Influence of Sulfadiazine on Plasma Lipids in Pneumonia.*

ALBERT V. STOESSER.

From the Department of Pediatrics, University of Minnesota, Minneapolis.

In preceding communications,[1,2] the author has presented evidence to indicate that in acute infections such as pneumonia in children, there is a definite depression of the plasma levels for total and ester cholesterol, total fatty acids and phospholipids together with a fall in the iodine numbers of the total fatty acids and the phospholipid fatty acids. Adequate treatment of the pneumonia patients with specific immune serum prevents the fall in blood lipids.[3] Chemotherapy offers a variable response. Sulfapyridine permits a moderate drop in the lipids of the blood while sulfathiazole is more effective.[4,5]

Sulfadiazine (2-sulfanilamidopyrimidine) is considered by clinicians to be superior to sulfathiazole in the treatment of pneumonia. What is its influence on the plasma lipids? This question is answered by the observations made on 6 subjects ranging in age from 6 to 13 years. All the children were ill with pneumonia due to the pneumococcus. They were admitted to the hospital very early during their illness, and on admission 2 g of sulfadiazine were given orally. Following the initial dose, the drug was administered in 1 g doses every 6 hours 4 times per day and it was well tolerated by all the patients.

The first sample of blood was collected just before the sulfadiazine was started. At this time each child had been ill from one to 2 days, and the fever ranged between 38.4° and 40.3°C. The second sample was drawn about 24 hours after the temperature had dropped to normal. The third and fourth samples were obtained on the fourth and seventh day of convalescence respectively. The drug was discontinued on the fourth day of normal temperature just before the third sample had been collected. A thorough examination including a roentgenogram of the lungs was made each time.

Bloor's methods[6,7,8] were followed to determine the various cholesterol fractions. The

TABLE I.
Plasma Lipids of Pneumonia Before, During, and After Administration of Sulfadiazine.

Case No.	Total cholesterol				Cholesterol esters, mg per 100 cc serum				Free cholesterol			
	A	B	C	D	A	B	C	D	A	B	C	D
1	170	275	297	287	74	193	203	184	96	82	94	103
2	131	198	190	223	74	139	121	152	57	59	69	71
3	180	193	190	208	110	112	116	127	70	81	74	81
4	147	260	208	242	81	186	146	160	66	74	62	82
5	145	197	256	260	62	122	167	169	83	75	89	91
6	136	253	193	247	56	155	117	156	80	98	76	91
Avg	151	230	239	244	76	151	145	158	75	78	77	86

Case No.	Total fatty acids, mg %				Iodine No. of total fatty acids				Phospholipids, mg %				Iodine No. of phospholipid fatty acids			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
1	385	420	417	405	105	97	99	106	130	93	125	147	116	112	112	115
2	264	252	296	364	102	104	98	93	104	92	91	120	101	112	107	106
3	350	375	366	334	108	103	112	106	93	139	119	119	117	111	118	116
4	268	283	420	405	96	104	99	108	104	92	125	120	101	112	112	106
5	280	346	380	413	101	103	107	110	130	139	113	130	104	111	108	109
6	319	452	366	381	96	105	112	97	130	99	119	123	106	115	118	106
Avg	311	354	374	383	101	102	104	103	115	109	115	126	107	112	112	109

A—Blood sample collected before sulfadiazine was started.
B— ” ” ” after 24 hours of normal temperature.
C— ” ” ” on 4th day of convalescent period.
D— ” ” ” on 7th day of convalescent period

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* This work was supported by a grant from the Medical Research Fund of the University of Minnesota.

[1] Stoesser, A. V., and McQuarrie, Irvine, *Am. J. Dis. Child.*, 1935, **49**, 658.

[2] Stoesser, A. V., *Am. J. Dis. Child.*, 1938, **56**, 1215.

[3] Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 168.

[4] Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 201.

[5] Stoesser, A. V., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 83.

[6] Bloor, W. R., *J. Biol. Chem.*, 1916, **24**, 227.

[7] Bloor, W. R., and Knudson, Arthur, *J. Biol. Chem.*, 1916, **27**, 107.

[8] Bloor, W. R., personal communication to the author.

total fatty acid and phospholipid values were obtained by the microgravimetric method of Wilson and Hansen.[9,10] Yasuda's modification[11] was employed to determine the iodine absorption of the serum fatty acids.

The results are summarized in Table 1.

The physical examinations and the roentgenograms of the lungs revealed a similar infection in all the subjects. The total cholesterol and esters were significantly lower than normal on the day the sulfadiazine was started. The only exception was case 3 which was the least ill. The response to the administration of the drug was rapid. Four of the patients had a precipitous drop in the temperature to normal within 24 hours while the remaining 2 had normal temperatures at the end of 36 hours. One day later the cholesterol esters already had risen to within the normal range leading to normal total cholesterol values. The roentgenograms showed beginning resolution. Blood examinations on the fourth day of convalescence revealed that the high levels of the various cholesterol fractions were being sustained. Resolution now was complete in all cases.

The influence of an acute infection on the total fatty acids and the phospholipids is less rapid than on the total cholesterol and esters. As a result the response to sulfadiazine therapy was prompt enough to prevent any significant change. The iodine numbers of the total fatty acids and of the phospholipid fatty acids remained practically the same during the entire course of the pneumonia.

The early use of sulfadiazine tends to control the altered lipid metabolism of acute infections. Comparing this observation with those of preceding papers[3,4,5] the drug is found to be as good as specific immune serum, much better than sulfapyridine and perceptibly more effective than sulfathiazole.

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[9] Wilson, W. R., and Hansen, A. E., *J. Biol. Chem.*, 1936, **112**, 457.

[10] Hansen, A. E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 376.

[11] Yasuda, M., *J. Biol. Chem.*, 1931-32, **94**, 401.

14558 P

Spontaneous Glomerulonephritis in Mice.*

ARTHUR KIRSCHBAUM.

From the Department of Anatomy, University of Minnesota Medical School, Minneapolis.

Although the clinical signs and symptoms, morphologic pathology, and the chemical alterations of the blood and body fluids are known in glomerulonephritis, investigations on etiology and pathogenesis have been retarded because experimental material has not been available. Spontaneous glomerulonephritis in animals has not been described; the disease has been induced in rats according to several investigators.[1,2,3]

When mice of the Strong NH strain developed anasarca, ascites, and hydrothorax spontaneously, and became dyspnoeic, the condition was considered worthy of detailed study. Eight edematous animals of this strain were investigated from the morphologic standpoint. The kidneys were pale, but of approximately normal size and weight. On histologic section they revealed glomeruli with alterations strikingly similar to those seen in human chronic

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* This investigation was aided by grants from the Research Funds of the Graduate School of the University of Minnesota and The Jane Coffin Childs Memorial Fund for Medical Research. The author is indebted to Dr. E. T. Bell, Professor of Pathology in the University of Minnesota Medical School, who examined the kidney sections and provided facilities for special staining procedures.

[1] Masugi, M., and Sato, Y., *Virch. Arch. path. Anat.*, 1934, **293**, 615.

[2] Arnott, W. M., Kellar, R. J., and Matthew, G. D., *Edinb. Med. J.*, 1936, **43**, 233.

[3] Smadel, J. E., and Swift, H. F., *J. Exp. Med.*, 1941, **74**, 345.

glomerulonephritis[4]—hyalinized glomeruli, and glomerular closure due to endothelial proliferation. These features were elucidated by staining with azo-carmine. In some cases not all the glomeruli were involved, whereas in others the process was general enough so that the term "diffuse glomerulonephritis" was applicable. Albuminuria was evidenced by casts in the tubules, but only a small degree of tubular degenerative change was present. Contracted kidneys have not been observed.

Preliminary chemical study has confirmed the morphologic diagnosis. Albuminuria, decreased total plasma proteins (5.0 g as compared with a normal for the mouse of 6.2-6.5 g %), and hyperlipemia have been demonstrated for one typical case.

The age of clinical appearance of the disease, when associated with edema, has ranged from 5 to 18 months; death ensued within 2 to 3 weeks in most cases after the development of massive edema. Glomerular pathology has been noted in animals which did not exhibit the nephrotic tendency. The incidence of the disease in untreated strain NH mice of the Minnesota stock has not been determined.† Further studies on the pathology of the disease in mice are being pursued; investigations on the genetic, chemical, bacteriologic, and nutritional aspects of the problem are being planned.

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[4] Bell, E. T., *Am. J. Path.*, 1938, **14**, 691.

† The animals from which the Minnesota stock of the NH strain has been derived were obtained from Dr. L. C. Strong of the Yale University School of Medicine.

14559

Hormonal Influences on Chemically-Induced Tumors of the Reproductive System.

STEPHEN A. CASTELLANO AND FRED E. D'AMOUR.
From the Biologic Research Laboratories, University of Denver.

In a recent study, Smith and associates[1] reported that a deficiency or excess of numerous hormones had, with few exceptions, little effect on the rate of development of chemically-induced tumors. In that study methylcholanthrene was introduced subcutaneously, in the present investigation the same carcinogen was placed directly into various organs of the reproductive system and attention centered upon gonadal and gonadotropic hormones as possible influencing agents.

Experimental. University of Denver strain white rats, whose spontaneous tumor incidence is practically zero, were employed. They were 3 months old at the time of carcinogen administration, those surviving were killed one year later. Small pellets of methylcholanthrene, in 20% suspension in paraffin, were introduced, under aseptic precautions, into the organ studied, the total dose per animal being 10 mg. All animals were examined carefully 3 times weekly, the date upon which a tumor of palpable size first was noted was recorded; if within a week it was apparent that the mass had increased in size (which was usually the case) the original date was accepted. If no growth ensued the site was directly inspected and a decision reached. Autopsy of the survivors at year's end revealed no tumors not previously diagnosed and the implanted carcinogen was found to have been retained in all animals which had not developed tumors.

Results. Tables I and II present the results obtained. The dosage of hormones shown is the amount given per injection. Injections were made 3 times weekly.*

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[1] Smith, Donn L., Wells, J. A., and D'Amour, F. E., *Cancer Research*, 1942, **2**, 40.

* Our thanks are herewith expressed to the following companies for the generous amounts of hormone preparations supplied: G. W. Carnrick Co., Ciba Pharmaceutical Products, Inc., Parke, Davis and Co., The Schering Corporation, The Upjohn Co., and the Winthrop Chemical Co., Inc.

TABLE I.
Effect of Hormones upon Tumor Development.
Females.

Group	No. rats	Treatment	No. developing tumors	Avg time, days
A	10	Paraffin in Uterus	0	—
B	20	Methylcholanthrene in Uterus	2	180
C	10	Castrate; M.C. in Uterus	1	257
D	9	M.C. in Uterus, plus Stilb., 5 γ 3× weekly	6	179
E	10	M.C. in Uterus, plus Stilb., 15 γ 3× weekly	7	173
F	10	M.C. in Uterus, plus Progestin, 5 mg, 3× weekly	1	192
G	10	M.C. in Ut., plus Stilb. 5 γ and Progestin 5 mg, 3× weekly	1	226
H	10	M.C. in Uterus, plus P.U. gonadotropin, 1 I.U. 3× weekly	1	251
I	10	M.C. in Uterus, plus P.M.S. gonadotropin, 1 I.U. 3× weekly	3	140
J	10	M.C. in Ovaries	0	—
K	10	M.C. in Ovaries, plus P.M.S. gonadotropin, 1 I.U. 3× weekly	1	51

TABLE II.
Males.

Group	No. rats	Treatment	No. developing tumors	Avg time, days
L	10	Methylcholanthrene in Testes	0	—
M	10	M.C. in Testes, plus P.U. gonadotropin, 1 I.U. 3× weekly	0	—
N	10	M.C. in Testes, plus P.M.S. gonadotropin, 1 I.U. 3× weekly	0	—
O	10	M.C. in Testes, plus Testosterone, 20 γ 3× weekly	0	—
P	10	M.C. in Sem. Ves. and Prostate	3*	305
Q	10	Castrate; M.C. in Sem. Ves. and Prostate	10†	254
R	10	M.C. in Sem. Ves. and Prostate plus Testosterone, 20 γ 3× weekly	5‡	230

* Two tumors in prostate alone, 1 in seminal vesicle alone.

† Three tumors in prostate alone, 7 in both seminal vesicles and prostate.

‡ All 5 in prostate alone.

Discussion. Even under the stimulus of gonadotropic hormones, the gonads themselves appear to be highly resistant to the carcinogenic action of methylcholanthrene. Only one ovarian tumor developed in 20 females (groups J and K), and no testicular tumors in 40 males (groups L to O).

The stimulating action of estrogens upon some types of uterine tumors appears to be well established. Our results support this view as applying to tumors induced by methylcholanthrene, the incidence ranging from 10% in ovariectomized rats to 70% in the group receiving 45 γ of stilbestrol per week. It is interesting that progestin appears to inhibit this action, as is shown by comparing groups D and G.

In the paper previously mentioned, Smith reported a statistically significant hastening of the appearance of subcutaneous tumors under the influence of P.U. and P.M.S. gonadotropins, especially the latter. For P.M.S., this finding appears to be duplicated in the present study; the 4 tumors produced under its influence (groups I and K) having distinctly shorter incubation periods.

As regards the effect of testosterone on tumors of the male adnexæ, our results are contradictory. The animals having the lowest concentration, namely, the castrate group, had an incidence of 100% tumors, those receiving testosterone, 50%, and the normals, 30%. In view of the present trend toward castration in treating prostatic tumors in the human it would be interesting to see what effect castration would have if done after, rather than before, the development of the tumor. This point, as well as other inconsistencies in the male groups is now being investigated with larger numbers of animals.

Summary. An attempt was made to influence the development of methylcholanthrene-induced tumors of the reproductive system by means of gonadal and gonadotropic hormones. The results follow: 1. The gonads themselves were found to be highly resistant to

the carcinogenic action of methylcholanthrene. 2. Administration of stilbestrol increased the incidence of uterine tumors. 3. The effect of testosterone upon tumors of the male adnexæ was contradictory.

14560

An Ether-Extractable Substance from Blood Serum and Buffy Coat Which Contracts Smooth Muscle.

CHIAO TSAI, JAMES J. MCBRIDE, AND MARJORIE B. ZUCKER. (Introduced by Magnus I. Gregersen.)

From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

The presence in blood serum and platelets of substances which contract smooth muscle has been recognized for the past seventy years. Although numerous investigators have prepared active extracts of serum and platelets by the use of alcohol and acetone, few have employed water-immiscible solvents. T. F. Zucker and Stewart[1] and Janeway *et al.*,[2] using strips of ox carotid artery as an indicator, reported that only a small fraction of the total activity of serum was extractable by ether. Recently, Wu, Fang, and Tsai[3] reported the separation of a fraction from dried rabbit serum by extraction with ether. This fraction was active on the rabbit intestine, but not on the vessels of the perfused rabbit ear, whereas rabbit serum was active on both of these indicators.* In the present paper, the properties of an ether extractable fraction which appears to be identical with that obtained by Wu *et al.*[3] will be described.

Methods. In the original method of Wu *et al.*,[3] the serum was dried on a steam-bath before extraction with ether. We were unable to obtain consistent results with this somewhat drastic method, but found that an ether extract made from serum dried at room temperature with Na_2SO_4 was uniformly active.

Rabbit serum was ground thoroughly with 5 times its weight of anhydrous Na_2SO_4. The powder was then extracted 4 times with dry ether and the ether was evaporated on a steam-bath from the combined extracts. Approximately 0.1 mg of residue was obtained from 1 cc of serum, of which about one-half was soluble in water. Extracts were prepared in a similar manner from cat and human serum. The portion of the residue soluble in Locke solution will be referred to as "extract of dry serum." Solutions were made up so that 1 cc of extract represented from 1 to 10 cc of the original serum. This made it possible to compare the relative activity of serum and extract because the activity of both could be made approximately equal. The dosage values given in Table I represent the volume, not of the extract, but of the serum from which it was obtained.

The activity of an extract of the buffy coat of human citrated blood was also studied. The buffy coat was mixed with an equal volume of acetone and filtered. The filtrate was freed of acetone by vacuum distillation. The resulting aqueous extract was dried by mixing

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[1] Zucker, T. F., and Stewart, G. N., *Zentralbl. f. Physiol.*, 1913, **27**, 85.

[2] Janeway, T. C., Richardson, H. B., and Park, E. A., *Arch. Int. Med.*, 1918, **21**, 565.

[3] Wu, C. H., Fang, H. S., and Tsai, C., *Proc. Chinese Physiol. Soc., Chengtu Branch*, 1942, **1**, 83.

* One of us (M.B.Z.), unaware of the work of Wu *et al.*,[3] had separated an active fraction from the buffy coat of human blood. The dry fraction was insoluble in ether. In doses of 0.5 to 2.0 mg it constricted the vessels of the perfused cat tail and stimulated the muscle of the rat and rabbit intestine. The recent visit of Dr. Tsai to this country presented an opportunity to compare the fractions studied in the Chinese and American laboratories. The present paper is a partial report of this joint study. Data on the fraction prepared by M.B.Z. and the comparison of the two fractions will be reported elsewhere.

TABLE I.

Comparison of the Action of Serum and Extracts of Dry Serum and Buffy Coat on Smooth Muscle.*

Material	Rat gut		Rabbit ear		Cat tail		Cat N.M.	
	Dose, cc	Effect	Dose, cc	Effect	Dose, cc	Effect	Dose, cc	Effect
Rabbit serum	0.04	++++	0.1	+++	0.1	++++	0.3	++++
Extr. dry rab. serum†	0.5	+++	2.0	0	1.0	++	2.0	++
Cat serum	0.04	++++	0.1	+++	0.1	++++	0.3	++++
Human serum	0.8	++	1.0	+	0.5	++	—	—
Extr. dry human buffy coat‡	0.4	+++	1.0	0	1.0	++	4.0	++++

* The data presented, with the exception of those concerning human serum, represent assays performed on the same indicators on a single day. They are typical of the results obtained in numerous experiments.

† The dosage represents the volume of serum from which the extract was prepared, not the volume of extract administered.

‡ One cc represents the extract from the buffy coat of 950 cc of blood.

it with 5 times its weight of Na_2SO_4, and the powder extracted with ether in the same manner as was the serum. One cc of the final extract represented the buffy coat of from 500 to 1200 cc of blood.

As test objects, rabbit ileum, rat duodenum, cat nictitating membrane, and the vessels of the perfused rabbit ear and the cat tail were used. The ear and tail were perfused at room temperature at a pressure of 30 to 70 cm H_2O. The intestine was suspended under slight tension in a 20 cc bath maintained at 37°C. The maximum volume of test solution used was 1 cc, except in the tests on the previously denervated nictitating membrane, in which the solution in a 2 cc volume was injected peripherally through the cannulated carotid artery. The tests on each preparation were repeated several times in a different order. Control tests of Locke solution and of a blank Na_2SO_4 extract were frequently made and were uniformly negative.

Results. The extract of dry rabbit serum had between one-tenth and one-twentieth of the activity of the original serum on the rat duodenum, cat nictitating membrane and tail vessels (Table I). The substance was active on the rat intestine in a dilution of 1:500,000. The rabbit ileum was less sensitive to both serum and extract than was the rat duodenum. The extract had no effect on the vessels of the perfused rabbit ear, even in a dose equivalent to 7 cc of serum, whereas 0.1 cc of whole rabbit serum had a marked constrictor action.

Extracts of dry human and cat serum also showed about one tenth of the activity of the original serum on the rat intestine, but their effect on the rabbit ear vessels was equivocal. The pharmacological properties of the extract of buffy coat were identical with those of the extract of dry rabbit serum.

It was found that an aqueous solution of the extract, when dried on a steam bath, yielded a residue insoluble in ether. This residue retained all of the activity of the original extract as tested on the rat intestine. It may be concluded, therefore, that the active material is soluble in ether only in the presence of certain of the lipids in serum and the buffy coat.

The active material in the extracts of dry serum and buffy coat resisted boiling in Locke solution for 10 minutes. The activity of these extracts, whether or not lipids were present, was markedly diminished by treatment with 0.1 N NaOH for ½ to 1½ hours at room temperature, whereas similar treatment with 0.1 N HCl had no effect.

Discussion. It should be noted that, although the extracts of rabbit serum and of human buffy coat stimulate the smooth muscle of the rat intestine, they have no effect upon the vessels of the rabbit ear. On the other hand, the ear vessels are strongly constricted by 0.1 γ of histamine,[4] whereas the rat intestine is very insensitive to this drug. These observations indicate that the active constituent in the ether extracts cannot be histamine.

Rabbit serum constricts the blood vessels of both the rabbit ear and the cat tail. The fact that the extract of dry serum does not act upon the vessels of the rabbit ear indicates that rabbit serum contains active substances not present in the extract. It is relevant to consider the nature of these substances. The histamine content of rabbit serum (1 to 3 γ/cc[5,6]) is so great that it may be responsible for a considerable part of the activity of serum on the rabbit ear vessels. Evidence for this was furnished by Tsai *et al.*[7] who found that incubation of rabbit serum with crude histaminase reduced its vasoconstrictor activity by about one-third. As these workers pointed out, the residual activity indicates the presence of a non-histamine vasoconstrictor substance in rabbit serum. Such a substance must be responsible for the major part of the activity of cat and human sera upon rabbit ear vessels, since these sera contain insufficient histamine (0 to 0.06 γ/cc[5,6,8]) to account for an appreciable part of their vasoconstrictor activity.

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[4] Rothlin, E., *Biochem. Z.*, 1920, **111**, 299.

Summary. A substance which stimulates smooth muscle was separated from dried rabbit serum and from the buffy coat of human blood by ether extraction. This substance is soluble in ether only in the presence of certain blood lipids. Aqueous solutions of the material stimulate the rat and rabbit intestine, cat nictitating membrane and tail vessels, but are devoid of effect on the vessels of the rabbit ear. The present findings, together with those of Tsai *et al.*,[7] suggest that several vasoconstrictor substances are present in serum.

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[5] Code, C. F., *J. Physiol.*, 1937, **90**, 349.

[6] Anrep, G. V., Barsoum, G. S., Talaat, M., and Wieninger, E., *J. Physiol.*, 1939, **96**, 130.

[7] Tsai, C., Wu, C. H., Fang, H. S., and Chen, T. I., *Proc. Chinese Physiol. Soc., Chengtu Branch*, 1943, **2**, 1.

[8] Barsoum, G. S., and Gaddum, J. H., *J. Physiol.*, 1935, **85**, 1.

14561

Further Studies on the Menstrual Discharge of Women.*

O. WATKINS SMITH AND GEORGE VAN S. SMITH.

From the Fearing Research Laboratory, Free Hospital for Women, Brookline, Mass.

Earlier studies on the menstrual discharge of normal women have been reported[1] with emphasis on its great toxicity, as little as 0.01 cc when fresh killing an immature rat within 48 hours. This paper summarizes certain corrections and additions that have evolved from continued investigation of this material.

It was stated that the toxin was most concentrated in the cells and debris of menstrual fluid. We have since found that the endometrial debris forms upon centrifugation, a middle layer between the serum and blood corpuscles. Even after being washed 3 to 6 times with saline, this layer is nearly 10 times as toxic as the serum, whereas the washed red blood cells are practically harmless. The amount of endometrial debris varies, being least when the rate of catamenial flow is greatest; the toxicity of different collections during menstruation, whether serum or whole discharge is tested, varies in direct proportion to the quantity of endometrial debris It would appear, therefore, that the toxicity is derived from the endometrial debris.

As stated, after fractional precipitation of whole discharge with $(NH_4)_2SO_4$ toxicity is most concentrated in the water-insoluble portion (after dialysis) of the englobulin precipitate. This precipitate, however, is apparently not a true englobulin, since it does not go into solution in the presence of electrolytes. When the whole discharge is not filtered before precipitation, the endometrial debris itself might be carried down and account for

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* The Mrs. William Lowell Putnam Investigation of the Toxemias of Pregnancy, aided by grants from the Committee for Research in Problems of Sex, National Research Council.

[1] Smith, O. W., and Smith, G. V., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 100.

the toxicity of this fraction. However, even when filtered serum from the discharge is ⅓ saturated with $(NH_4)_2SO_4$ and the precipitate dialysed, the resultant water-insoluble material is also insoluble in normal saline and lethal to immature rats in amounts equivalent to 0.1 to 2.0 cc of the original serum. Denaturation in the process of fractionation cannot be the explanation, since exactly the same technic applied to normal venous serum yields an englobulin fraction soluble in normal saline and non-toxic even in amounts equivalent to 10.0 to 15.0 cc of serum. After dialysis some of the englobulin precipitate from laked whole normal blood or from the serum of badly hemolysed or lipemic normal blood is insoluble in saline but does not harm immature rats in amounts equivalent to 5.0 to 10.0 cc of whole blood or serum. The fact that the serum of menstrual fluid almost invariably contains products of hemolysis, therefore, cannot account for its toxicity. Furthermore, whole discharge that has stood and become badly hemolysed is less toxic than when fresh and only slightly hemolysed. These observations strengthen our original intimation[1] that if the toxin is a decomposed protein it is formed *in vivo*. It may be a specific denaturation product of the endometrium consequent upon withdrawal of hormonal support.

Attempts to purify the toxin have been hampered by its lability. The minimal lethal dose before and after various procedures aimed towards stabilization and purification has been established by tests on 19- to 21-day-old male rats weighing 20 to 25 g, our criterion being death within 48 hours after the subcutaneous injection of a given amount. Although the whole discharge, rapidly dried, sealed and stored in the refrigerator, may retain its original potency for years, the toxicity of wet material, even when quickly frozen and stored at −18°C, may almost completely disappear overnight. Partially purified toxin is particularly labile and loses potency while being dried or soon thereafter. Our purest preparation to date is a grayish-white powder made from filtered serum which had been ⅓ saturated with $(NH_4)_2SO_4$. The precipitate was washed twice with ⅓ saturated $(NH_4)_2SO_4$ and dialysed until sulphate-free (24 hours in running tap water). The dialysed material was centrifuged, the precipitate washed once with normal saline and dry-frozen in a Flosdorf-Mudd apparatus. When first prepared, 5.0 mg (equivalent to 0.75 cc of serum) suspended in saline was the M.L.D.; at the last testing, 6 weeks later, 20.0 mg were required.

We reported that pretreatment with progesterone, adrenal cortical extract or the serum of rabbits immunized against menstrual discharge protects rats against lethal amounts of toxin. These therapies cannot be considered specific because of the surprising discovery that even water or normal saline renders an immature rat resistant to an otherwise lethal dose of menstrual fluid if as much as 1.0 cc is injected subcutaneously twice daily for 2 days prior to giving the toxin. This is, of course, a large volume of fluid for such a small animal (nearly ⅕ of the body weight). Does increased adrenal activity therefrom provide protection? Desoxycorticosterone or adrenal cortical extract given together with a M.L.D. of toxin does not prevent death but prolongs significantly the length of survival. Whatever the explanation, the finding indicates that susceptibility to the toxin is markedly influenced by the animal's condition as regards water balance. Our earlier observations on the influence of hormonal status upon susceptibility may perhaps all be interpreted on this basis, since an effect of steroid hormones on water balance has become well recognized.

It was also reported that serum from rabbits which had received repeated injections of sublethal doses would not protect against the toxin if simultaneously administered. We have found that adequate immunity was never established in these early experiments. Until a precipitin titer of 1 to 1,000,000 or over is established, no protective action is demonstrable. Unless small doses are given at first, that is, no more than the M.L.D. for an immature rat, the rabbit is likely to die. Even with this amount loss of appetite and listlessness are noted and, after 2 or 3 injections at 5-day intervals, severe subcutaneous reactions develop with diffuse and extensive edema and brawny induration.

With continued injections the local and systemic reactions become much less intense, even though the dose is doubled. The inflamed areas from the early injections, some of which have ulcerated, become abscesses which drain and heal and no new ones appear. By this time the precipitin titer is very high and the serum prolongs the period of survival of an immature rat when given simultaneously with a M.L.D. of menstrual toxin. Into one of 2 littermate rats 1.0 cc of rabbit serum is injected subcutaneously and into the other 1.0 cc of saline. One-half hour later 1.0 cc of serum and saline, respectively, is injected and, in a different site, a M.L.D. of menstrual toxin. In 7 such experiments, using serum from 5 immune rabbits, the rats treated with serum lived 6 to 48 hours longer than the saline-treated rats. The longest interval between deaths of littermate rats simultaneously receiving a M.L.D. of toxin alone has been 3 hours. Moreover, in 17 experiments similar to the above except that serum was taken from rabbits before injections of menstrual toxin were started or before adequate immunity was established, no rat injected with rabbit serum lived more than 3 hours longer than its saline-injected littermate. The fact that transferable immunity may be produced with menstrual toxin seems to be clear evidence of its specificity.

With the idea that a substance similar to or identical with the toxin of menstrual discharge might result from other forms of cellular injury, we have investigated the englobulin fraction of the blood of severely burned rats, degenerating livers of guinea pigs and rats and bleeding decidua from spayed pseudopregnant guinea pigs. Our object was to immunize rabbits against toxin from some source other than human, first, with the thought that by means of cross precipitin reactions we might have a test for circulating menstrual-like toxin in humans when it may be present in small amounts, viz., at the time of menstruation and in late pregnancy toxemia,[2] and conceivably in shock. Secondly, if immune serum were to be administered to humans it would, of course, be necessary to eliminate human proteins from the immunizing material. To date we have not succeeded either in finding anything similar to the menstrual toxin or in obtaining from rabbits immunized against these materials serum capable of precipitating menstrual toxin or of delaying the death of rats when given together with a M.L.D.[†] These negative results, in contrast to those with menstrual fluid, supply additional controls and thereby strengthen the evidence in favor of the specificity of the menstrual toxin.

Conclusion. The toxicity of the menstrual discharge of normal women appears to be due to a specific product of the endometrium formed *in vivo*.

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[2] Smith, G. V., and Smith, O. W., *J. Clin. Endocrinol.*, 1941, **1**, 470.

[†] While this work was in progress, Menkin reported the finding of an injurious factor in the englobulin fraction of inflammatory exudates of dogs and man which he named "necrosin."[3] From his description we surmised that his factor and our menstrual toxin might be identical. Investigation of this possibility is now under way.

[3] Menkin, V., *Science*, 1943, **97**, 165.

14562

Relation of Renin to Human Hypertension with Particular Reference to Eclampsia, Preeclampsia and Acute Glomerulonephritis.*

Lewis Dexter and Florence W. Haynes. (Introduced by J. H. Mueller.)

From the Medical Clinic, Peter Bent Brigham Hospital, and the Department of Medicine, Harvard Medical School, Boston.

The agent responsible for the rise of blood pressure resulting from constriction of the renal artery in experimental animals was first shown by Braun-Menendez *et al.*[1] to be due to the renal enzyme, renin, and their findings have been further extended[2] and confirmed.[3] The present study describes the assay of renin in the blood of patients with various types of hypertension.

Methods. Renin in human blood was determined by the following modification of the method of Leloir *et al.*[4] Fifty cc or more of venous blood, with or without an anticoagulant, were withdrawn from hypertensive patients and placed on ice immediately. After centrifugation, 0.09 g solid sodium chloride was added for each cc of serum or plasma. It was cooled to 5°C and the pH lowered to 2.0 (glass electrode) with 10% hydrochloric acid and left for 2 hours at this temperature in order to destroy hypertensinase. The pH was raised to about 6 with N sodium hydroxide and the solution dialyzed with a little toluol overnight against tap water at 5°C. It was then divided into 2 or, if sufficient amounts of blood had been obtained, 3 equal parts, each part representing about 10 cc of original serum or plasma. To each were added an excess (6 to 8 cc) of hypertensinase-free beef hypertensinogen,[4] 0.5 cc M/2 phosphate buffer at pH 7.3, and 0.2 cc 1:1000 merthiolate (Lilly). The first tube was incubated at 37°C for 4 hours; the second tube was unincubated; the third, which was often omitted, was incubated at 37°C for 4 hours with enough hemolyzed fresh human red blood cells (hypertensinase) to produce a pink color. At the end of incubation the tubes were heated in boiling water for 5 to 10 minutes at about pH 4. Three volumes of 95% ethyl alcohol were then added, the precipitated proteins removed by filtration, the filtrate made slightly acid, and the alcohol removed by evaporation under reduced pressure over boiling water to a volume of 2 or 3 cc. The contents of the flask were taken up in two 5 cc washings with normal saline, adjusted to neutral, and their content of hypertensin assayed in cats under dial anesthesia by the method of Leloir *et al.*[4]

The specificity of the method was indicated by a pressure rise from the intravenous injection of the first tube and a lack of pressor response from the second and third tubes.[4] Other pressor substances such as adrenalin, pituitrin, "spätgift," tyramine, and guanidine are not recovered by this method. The addition of 0.2 cat unit of human renin to human plasma can be recovered by this technic when assayed on a suitable animal preparation.

Results. Significant amounts of renin were found in the blood of 1 patient with eclampsia, 2 with severe preeclampsia, and 1 with fulminating acute glomerulonephritis without convulsions (see Table I and Fig. 1). In 3 cases the hypertension was of sudden onset. In 1 of the preeclamptics it was of more gradual onset, but was superimposed on a previous essential hypertension. The blood was obtained soon after admission to the hospital when the blood pressure was approaching its maximum elevation and before treatment was instituted. In the case of the 4-year-old boy, P.P., with acute glomerulo-

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* This investigation was aided in part by a grant from the John and Mary R. Markle Foundation.

[1] Braun-Menendez, E., Fasciolo, J. C., Leloir, L. F., and Muñoz, J. M., *Rev. Soc. argent. de biol.*, 1939, **15**, 420.

[2] Houssay, B. A., *Medicina*, 1941, **1**, 167.

[3] Kohlstaedt, K. G., and Page, I. H., Proc. Soc. Exp. Biol. and Med., 1940, **43**, 136.

[4] Leloir, L. F., Muñoz, J. M., Braun-Menendez, E., and Fasciolo, J. C., *Rev. Soc. argent. de biol.*, 1940, **16**, 635.

TABLE I.
Amount of Renin Recovered from Blood of 3 Patients with Toxemia of Pregnancy and 1 with Acute Glomerulonephritis.

Patient	Blood pressure, mm Hg.	Amt serum tested, cc	Cat units renin recovered	Diagnosis and remarks
P.L.	160/110	11	0.4	Eclampsia
I.D.	158/118	11	0.7	Severe preeclampsia.
L.D.	180/110	9	0.5	Essential hypertension with preeclampsia.
P.P.	248/160	10	2.4	Acute glomerulonephritis, fulminating. B.P. 85/50 24 hrs previously.

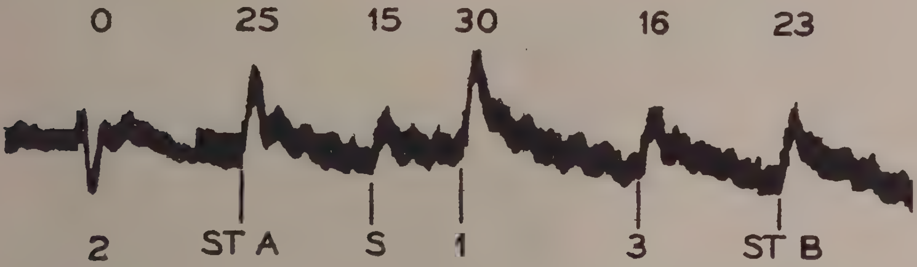

FIG. 1.
Demonstration of Renin in the Plasma of Patient P.P. with Acute Glomerulonephritis. Blood Pressure: 248/160 mm Hg.

Blood pressure curves are those of a cat under dial anesthesia.

The figures above the curves indicate the rise in blood pressure in mm Hg.

2—Response to injection of unincubated control equivalent to 10 cc serum. (See text for preparation of tubes 1, 2, and 3.) After this injection the cannula clotted.

St. A—Response to 0.75 cat unit of standard hypertensin.

S—Response to 10 cc physiological saline.

1—Response to incubated sample equivalent to 5 cc serum. When calculated for renin, this is equivalent to 2.4 cat units of renin per 10 cc serum.

3—Response to sample incubated with hypertensinase. Note that this gives approximately the same rise as the saline control.

St. B—Response to 1.2 cat units of standard hypertensin.

nephritis, which was verified subsequently by autopsy, a therapeutic phlebotomy made possible duplicate determinations of renin in the serum. Renin could be demonstrated in all tests, and large amounts of hypertensinase were necessary to destroy the hypertensin formed. In 5 cases of preeclampsia and in 2 of acute glomerulonephritis where the hypertension was not severe or where it was gradual in onset, no renin was detected in the blood. In 13 patients with chronic hypertension of all degrees of severity associated with chronic glomerulonephritis, chronic pyelonephritis, or of unknown cause (essential hypertension), no renin was found in the blood (Fig. 2).

Discussion. Dell'Oro and Braun-Menendez,[5] using the method of Leloir *et al.*,[4] have shown that renin may be detected in the systemic blood of dogs made hypertensive by means of a Goldblatt clamp during the time when the blood pressure is rising abruptly. Once the pressure has attained its maximum and plateaued, renin is not demonstrable in the systemic blood by this method. It has been claimed that renin can be detected in the blood of hypertensive patients using the isolated rabbit's ear[6] and the toad's hind limb,[7] but the technics as described made little attempt to identify the nature of the constrictor substance. It has only been by the

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[5] Dell'Oro, E., and Braun-Menendez, E., *Rev. Soc. argent. de biol.*, 1942, **18**, 65.

[6] Page, I. H., *J. Exp. Med.*, 1940, **72**, 301.

[7] Garreton Silva, A., Croxatto, R., Fuenzalida, O., and Viveros, R., *Rev. med. de Chile*, 1941, **69**, 625.

[8] Houssay, B. A., and Fasciolo, J. C., *Rev. Soc. argent. de biol.*, 1937, **13**, 284.

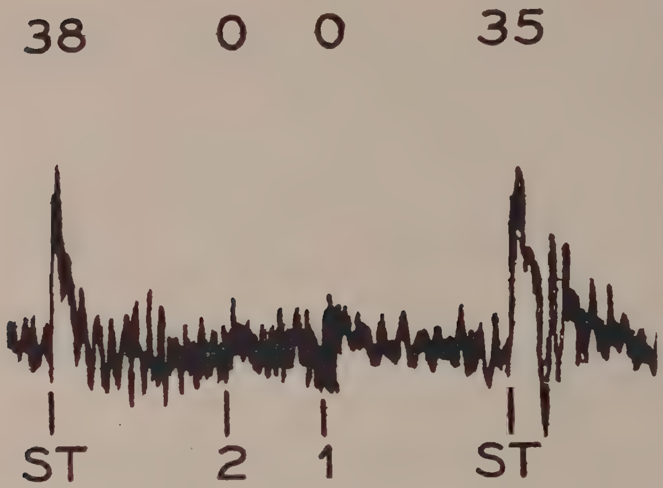

FIG. 2.

Test for the Presence of Renin in the Plasma of Patient B.F. with Essential Hypertension. Blood Pressure: 195/110 mm Hg.

Blood pressure curves are those of a cat under dial anesthesia.

The figures above the curves indicate the rise in blood pressure in mm Hg.

St.—Response to 0.5 cat unit of standard hypertensin.

2—Response to unincubated control equivalent to 10.5 cc plasma. (See text for preparation of tubes 1 and 2.)

1—Response to incubated sample equivalent to 10.5 cc plasma.

N.B. Renin was therefore not demonstrated in the blood of this patient.

use of kidney grafts[8] and cross-circulation experiments[9] that a pressor substance, similar in its pharmacologic properties to renin, has been demonstrated unequivocally in the systemic blood of dogs with chronic Goldblatt hypertension.

In patients we have found renin in the blood in those cases where the hypertension has been severe and explosive in onset, *i.e.*, eclampsia, severe preeclampsia, and fulminating acute glomerulonephritis. In cases of preeclampsia and acute glomerulonephritis with only moderate elevation of blood pressure or where the hypertension has been of gradual onset, and in patients with chronic hypertension with pressures as high as 285 systolic and 180 diastolic, we have been unable to detect renin in the systemic blood.

Summary. 1. Significant amounts of renin have been detected in the blood of 1 patient with eclampsia, 2 with severe preeclampsia, and 1 with fulminating acute glomerulonephritis using a modification of the method of Leloir *et al.*[4] 2. No renin has been found in the blood of patients with milder degrees of toxemia of pregnancy and acute glomerulonephritis, nor of patients with chronic hypertension of all degrees of severity. 3. The relation of these observations to those on animals with experimental renal hypertension is discussed.

We are indebted to Dr. Frederick L. Good of the Obstetrical Service of the Boston City Hospital, Dr. Frederick C. Irving, Obstetrician-in-Chief of the Boston Lying-In Hospital, and Dr. Richard M. Smith of the Children's Hospital for their kindness in allowing us to study their patients.

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[9] Solandt, D. Y., Nassim, R., and Cowan, C. R., *Lancet*, 1940, **1**, 873.

14563

Anticephalin Activity and the Prothrombin Conversion Rate of Normal and Hemophilic Plasmas.

LEANDRO M. TOCANTINS.

From the Department of Medicine, Jefferson Medical College and Hospital, Philadelphia.

Important differences have been observed in the results of the quantitative determination of prothrombin by the 1-stage[1] and 2-stage[2] methods. One explanation offered for these differences has been that the 1 stage method measures, not so much the total amount of prothrombin present but the speed with which prothrombin can be converted to thrombin and its titer built up to the clotting level.[3] In the 2-stage method, an excess of thromboplastin is added to the *diluted* plasma and enough time is allowed for the complete conversion of all the prothrombin into thrombin before fibrinogen is added and the clotting time measured. Dilution of the plasma is intended principally to overcome the action of inhibitors. It has not been clear, however, just what inhibitor, if any, is responsible for slowing the prothrombin conversion rate. Moreover, demonstrations of increases in the rapidity of this rate have not been sufficiently convincing to be generally accepted.[4]

The evidence to be presented seems to indicate that the prothrombin conversion rate is influenced by the anticephalin activity of the plasma. When anticephalin is present in excess (as in hemophilic plasma)[5] the rate of conversion is slow, but since in both the 1-stage and 2-stage methods a great excess of thromboplastin is used, and (in the 2-stage method) enough time is allowed for full conversion, little or no difference will be found between the results obtained by the two methods. If anticephalin has been reduced or removed, however, the rate of conversion of the remaining prothrombin is so accelerated as to lead to a pronounced difference in the results, the 1-stage method nearly always yielding higher values.

Plasma from normal and hemophilic men collected with especial precautions[6] was used either in the intact state or after exposure to adsorbents. Anticephalin may be rapidly removed from plasma by contact with kaolin, infusorial earth, pumice stone, asbestos fibers, aluminum hydroxide, etc.[7,8] Asbestos (short fibers, A. H. Thomas Co., Phila.) was used because of the ease with which the plasma can be separated from this adsorbent after the period of contact is over. By carefully adjusting the quantities of plasma and adsorbent, the period of contact and the temperature, it is possible to remove most of the anticephalin with only a slight loss in prothrombin and fibrinogen. By "adsorbed plasma" will be meant normal or hemophilic human citrated plasma that has been in contact with asbestos fibers (10 mg asbestos for each ml of plasma) for 2 hours at 20°C in a stoppered paraffin coated tube. The prothrombin and antithrombin content of the intact normal and hemophilic plasmas were within normal limits. The asbestos adsorbed plasma does not seem to lose antithrombin, but it contains slightly less prothrombin and fibrinogen than the intact plasma.

Adsorbed hemophilic plasma becomes unusually susceptible to activation by cephalin and cannot be distinguished in this respect from adsorbed normal plasma (Table I). As will be shown elsewhere, the response of a

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[1] Quick, A. J., Stanley Brown, M., and Bancroft, F. W., *Am. J. Med. Sci.*, 1935, **190**, 501.

[2] Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.

[3] Smith, H. P., Public. No. 13, Am. Assn. for Adv. of Sci., p. 10.

[4] Quick, A. J., *Hemorrhagic Diseases and the Physiology of Hemostasis*, 1942, p. 35.

[5] Tocantins, L. M., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 94.

[6] Tocantins, L. M., *Am. J. Physiol.*, 1943, **139**, 265.

[7] Tocantins, L. M., *Fed. Proc.*, 1943, **2**, 48.

[8] Tocantins, L. M., *Fed. Proc.*, 1944, in press.

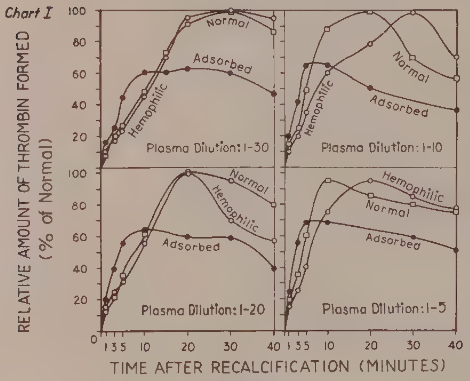
TABLE I.
Effect of Adsorption on Cephalin and Prothrombin Times of Normal and Hemophilic Plasmas.

	Normal		Hemophilic	
	Intact	Adsorbed	Intact	Adsorbed
Cephalin clotting time*				
0′ incubation (sec.)	84	32	177	31
5′ ” ”	97	30	210	31
Prothrombin time				
1-stage method† (sec)	12	8	12	9
2- ” ” ‡ ”	16	21	16	20

* 0.3 cc plasma, 0.1 cc cephalin (0 or 5 min. incubation), 0.1 cc 0.074 M $CaCl_2$.[5]

† Quick Method.[1]

‡ Smith, Warner and Brinkhous method,[2] using brain instead of lung extract and allowing 3 min. for conversion of the prothrombin.



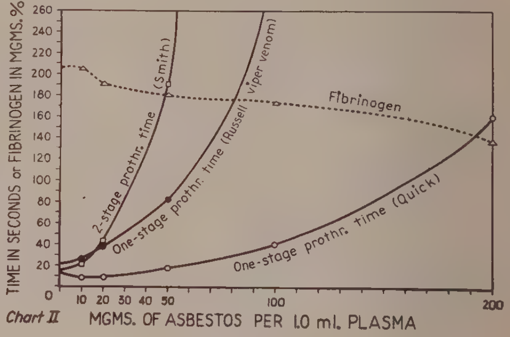


CHART I.

The rate of thrombin formation in 3 types of plasma, using cephalin. The shortest clotting time obtained with each dilution on normal plasma was considered to represent 100% thrombin formation for that specimen of plasma. The times equivalent to further dilutions (75%, 50%, etc.) of that plasma were determined and a curve plotted out. The percent of thrombin formed by that and the other plasmas at any period after recalcification was then calculated from their respective clotting times. Clotting mixtures as in Table II.

CHART II.

Effect on prothrombin time (3 methods) and fibrinogen of exposure of 1 ml of normal plasma to various amounts of asbestos for 60 min at 20°C. Prothrombin-free fibrinogen may be prepared in this manner, as the starting step.

given plasma to activation by cephalin may serve as an indication of its anticephalin (or antithromboplastin) activity provided its prothrombin is not under 50% of normal and there is no excess of antithrombin.

The adsorbed plasmas display shorter than normal prothrombin times by the 1-stage method but a longer time when tested by the 2-stage method (Table I). That this disagreement is brought about by an increase in the conversion rate of the adsorbed plasma prothrombin is shown in Chart 1 and Table II, In low dilutions, conversion of prothrombin by cephalin is slowest in the plasma with the most anticephalin activity (hemophilic) and fastest in that with the least (adsorbed). Diluting the plasma 1-20 or more weakens the inhibitor, thereby effacing most of the difference in anticephalin activity between hemophilic and normal plasma and tending to equalize their prothrombin conversion rates. Higher dilutions (1-80, 1-100), however, are necessary to reduce the anticephalin activity of the normal plasma to the level of that of the adsorbed plasma, before equalization of the prothrombin conversion rate of these two plasmas is brought about. When a potent thromboplastin solution instead of

TABLE II.
The Prothrombin Conversion Rate of Undiluted and Diluted Intact and Adsorbed Normal Plasma, Using Thromboplastin.

Type of plasma	Time (sec.) after recalcification before addition of fibrinogen.*										Dilut. of Plasma†
	0	4	10	15	20	30	60	120	180	300	
	Clotting time in seconds										
Intact	18	11	8	4	2						0
Adsorb.	13	7	4	2							0
Intact	34	26	21	19	13	11	9	9	9	9	1:5
Adsorb.	20	15	12	11	11	11	11	12	12	13	1:5
Intact	55		43		32	29	18	16	16	17	1:20
Adsorb.	47		38		32	29	22	22	22	25	1:20

* Contents of each tube: 0.3 cc incubation mixture ($CaCl_2$, 0.85% NaCl, thromboplastin, imidazole buffer),[2] 0.1 cc plasma, incubation for 0, 4, 10, etc., sec., then 0.1 cc fibrinogen.

† Defibrinated by addition of thrombin.

cephalin is employed (Table II), similar differences in the prothrombin conversion rate between normal and adsorbed plasmas may be observed, though lower dilutions of plasma must be used. The undiluted adsorbed plasma builds up a clotting titer of thrombin faster than the intact plasma and thus gives the impression that it has more prothrombin. When both plasmas are diluted 1-5 or 1-20, however, and enough time is allowed for the full conversion of all the prothrombin, the intact plasma gives a higher yield of thrombin, though, even at the high dilution, the adsorbed plasma still shows a slightly faster prothrombin conversion rate.

Exposure of 1 ml of plasma to quantities of the adsorbent greater than 10 mg intensifies the disagreement between the two methods (Chart II). When Russell viper venom, however, is used instead of brain extract, in the 1-stage method, the results are more in agreement with those obtained with the 2-stage method. Variations in prothrombin conversion rate apparently do not influence the action of Russell viper venom, probably because this venom acts directly on prothrombin, and is invulnerable to anticephalin.[7] The variations in conversion rate that have been pointed out for the prothrombin of various species[9] may be due to differences in anticephalin activity, in addition to chemical differences in the prothrombin itself. A study of the various animal prothrombins after exposure to adsorbents to remove anticephalin, may throw some light on this point.

Summary. Contact with certain adsorbents reduces the anticephalin activity of normal and hemophilic plasmas and accelerates the conversion rate of the prothrombin of such plasmas. Increases in the rate of conversion of prothrombin appear to be due to diminution of anticephalin activity and may be one cause of the disagreement in the results of quantitative determination of prothrombin by the 1-stage and 2-stage methods.

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[9] Warner, E. D., Brinkhous, K. M., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1940, **40**, 197.

14564

Vascular Pathology in Rabbits Following Administration of Foreign Protein.

ROBERT A. FOX AND LLOYD R. JONES.

From the Departments of Pathology and Bacteriology, St. Louis University School of Medicine, St. Louis.

During the past 9 months 3 lots of albino male and female rabbits (31 in all), weighing 1 to 3 kg each, were injected parenterally with horse serum. Each animal received one, 2, or 3 large doses (10 ml/kg) of serum administered either intravenously, intraperitoneally, or subcutaneously, and one or more smaller doses (1 ml), intravenously. Most of the animals were tested for skin hypersensitivity on one or more occasions. The total number of doses, the time interval between them, the sequence of large and small doses, and the time of skin testing were arbitrarily chosen, varied considerably, and seemed to bear no significant relationship to the observed results. For this reason a detailed record of each animal is deemed unnecessary. Those rabbits which did not die of anaphylaxis were sacrificed at arbitrary intervals following the last injection of serum. An autopsy was performed on all animals, and sections from a variety of organs were examined microscopically. Of the group of 31 rabbits, 20 exhibited pathologic changes of sufficient intensity to be considered significant. Seven showed changes which were considered to be only minimum in character and the remaining 4 were negative.

The significant histopathologic changes, which were limited almost exclusively to the heart, consisted primarily of mild vascular and perivascular alterations of the smaller branches of the coronary arteries. A few animals showed mild infiltration of leucocytes (mostly eosinophils) in the myocardium. Vascular and perivascular lesions were occasionally noted in the liver, lung, testis, kidney, and mesentery.

The vascular changes observed in the heart were characterized by intimal hyperplasia and proliferation of adventitial cells. The latter were frequently interspersed with, or surrounded by, lymphocytes and mononuclear cells, with an occasional polymorphonuclear neutrophil. Fibrinoid degeneration of the intima and media was noted infrequently, and necrosis was not observed. The mononuclear cells varied in size from medium to large, and often were multinucleated. They frequently occupied a paravascular position, and, intermingled with lymphocytes, adventitial cells and a few polymorphonuclear neutrophiles, vaguely resembled Aschoff bodies. The vascular lesions in which the cells were more circumferentially placed, resembled those often described as "rheumatic arteritis."

Klinge[1] and Vaubel[2] described similar vascular lesions in rabbit hearts following subcutaneous and intravenous injections of horse serum. Vaubel varied the size of the dose from small (0.005 ml) to large (30.0 ml), and observed alterations only in those animals which received 9 or more 2 to 10 ml injections. He was impressed by the similarity of the lesions to those of periarteritis nodosa, thromboangiitis obliterans, and to those noted in the necrotic portion of an Arthus skin reaction, and above all, to those of "rheumatic arteritis." He referred to the reaction as an "hyperergic arteritis." Klinge, on the other hand, was interested more in the pathologic changes in joints than in the changes which he observed in the heart muscle and valves. It was his opinion that the morphologic changes in the "blood vessel connective tissue apparatus" (perivascular tissue) and musculature (myocardium) closely resembled those observed in "human rheumatism" (rheumatic fever?). Recently Rich and Gregory observed similar lesions following intravenous injections of horse serum. The latter authors felt they had produced experimental periarteritis nodosa,[3] and "lesions with the basic characteristics of rheumatic carditis."[4]

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[1] Klinge, F., *Beitr. z. Path. Anat. u. z. allegem. Path.*, 1929, **83**, 185.

[2] Vaubel, E., *Beitr. z. Path. Anat. u. z. allegem. Path.*, 1932, **89**, 374.

On the basis of the following observations: (1) general well-being of the animals throughout the course of the experiment, (2) vascular lesions mainly in the heart, (3) general preservation of vascular architecture, (4) absence of nodular lesions in arteries, (5) absence of vascular necrosis, and (6) paucity of polymorphonuclear neutrophiles, and mild character of the vascular reaction in general, the lesions which we observed were considered to be unlike those of periarteritis nodosa in man. On the other hand, they correspond rather to those reported by Vaubel[1] as "rheumatic arteritis" and by Rich and Gregory[4] as "rheumatic carditis."

Certain immunological aspects of these experimental animals were studied in relation to the development of the vascular abnormalities. Of 23 rabbits, skin-tested during the course of the experimental work, all manifested positive reactions, as judged by inflammation, swelling, erythema, and induration at the site of injection. The more severe reactions in this group were characterized further by the development of grossly recognizable necrosis. This feature was observed in 13 of the animals, and these exhibited vascular alterations in the heart. This suggests that the degree of hypersensitivity requisite for the development of a necrotic type of skin reaction may be correlated in some manner with the capacity to develop vascular changes in the viscera. It is to be noted, however, that vascular reactions were also observed in 8 rabbits of the 10 which did not manifest the necrotic type of skin reaction. In this group the last skin test was made 8 to 17 days prior to necropsy, and while this interval does not differ materially from that in the previous group, it is recognized that the precise chronological relationship obtaining between marked skin hypersensitivity and the vascular allergic response has not been investigated. Furthermore, the potentiality for the necrotic type of skin reaction, as well as the vascular alteration itself, may be transitory in character and easily missed.

Twelve (39%) of the 31 rabbits developed the serum sickness reaction[5] on the ears, following a large parenteral dose of horse serum. A possible correlation between this reaction and pathologic changes in blood vessels is suggested, since all of the animals which developed the former were also found to have the latter.

Anaphylactic distress (mild or severe) was noted in 9 of 29 rabbits receiving two or more, spaced, parenteral injections of the antigen, and was not correlated with the development of morphologic vascular changes.

A correlation is known to exist between the capacity of the rabbit to develop the Arthus phenomenon upon intradermal administration of homologous antigen, and the humoral antibody level.[6] In our experiments it was noted that the capacity to develop a necrotic type of skin reaction was related to the development of pathologic changes in blood vessels; therefore, it is probable that further investigation will reveal quantitative correspondence also between humoral antibody levels and vascular changes.

Some of our rabbits exhibited vascular abnormalities following a single large dose of foreign serum, as was also noted by Rich and Gregory.[3] Added interest attaches to this observation because of its possible importance in relation to the clinical use of large doses of antisera and to the contemplated use of heterologous plasma, or its fractions, in the treatment of shock, etc., in man.

Summary. Mild vascular and perivascular alterations of the smaller branches of the coronary arteries were observed in a majority of rabbits following the parenteral injection of horse serum. No rabbit which elicited a necrotic-type of skin reaction failed to show vascular lesions.

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[3] Rich, A. R., and Gregory, J. E., *Bull. Johns Hop. Hosp.*, 1943, **72**, 65.

[4] Rich, A. R., and Gregory, J. E., *Bull. Johns Hop. Hosp.*, 1943, **73**, 239.

[5] Fleisher, M. S., and Jones, L., *J. Exp. Med.*, 1931, **54**, 597.

[6] Cannon, P. R., and Marshall, C. E., *J. Immunol.*, 1941, **40**, 127.

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